Tissue Distribution of C^{14}-labeled Beta-phenethylbiguanide

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Since the original report by Ungar et al.[1] on the hypoglycemic action of phenethylbiguanide, now more commonly known as DBI, the in vitro effects of this drug have been extensively studied. Under certain experimental conditions it can be demonstrated that the drug will inhibit in a variety of tissues the oxidation of glucose as well as other metabolic fuels.[2,3] At the same time tissue anoxia is produced presumably by the drug's action on the electron transport system.

Some of the in vitro effects of this drug have been suggested to explain its action in vivo; however, in our opinion, these studies have failed to yield any clear-cut information as to how this drug lowers the blood sugar in diabetic animals. A summary of the experimental observations has been reviewed recently by Williams and Steiner.[4]

In an attempt to shed some light on its hypoglycemic mechanism, we have labeled phenethylbiguanide with C^{14} and have conducted a time study of the urinary and body tissue distribution of the C^{14} label.

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The papers are arranged in the order of their presentation and the panel discussions or a summary of such panel discussions follows each group of related papers.

DBI is the U. S. Vitamin & Pharmaceutical Corporation brand name for beta-phenethylbiguanide hydrochloride. The compound also is known as beta-phenethylformamidinyliminourea hydrochloride, and as phenformin. These terms are used synonymously throughout the issue.

EXPERIMENTAL

Synthesis of radioactive phenethylbiguanide†-benzyl cyanide-C^{14}: Benzyl cyanide-C^{14} was prepared by the reaction of an excess of benzyl chloride with sodium cyanide-C^{14} (4.5 gm.) according to the procedure of Adams and Thal.[5]

β *phenethylamine-C^{14} hydrochloride*: Benzyl cyanide-C^{14} (5 gm.) was dissolved in dry ether (100 ml.) and added dropwise, with stirring, to a slurry of lithium aluminum hydride and dry ether (5 gm. $LiAlH_4$ in 300 ml. of dry ether). After addition of the benzyl cyanide (forty-five minutes) the mixture was refluxed for a further two hours. The excess lithium aluminum hydride was then destroyed by the cautious addition of water and the mixture acidified with 10 N sulfuric acid. The acidified aqueous solution was thoroughly mixed with the remaining ether layer. The ether layer was then separated and discarded, and the aqueous layer twice extracted with 100 ml. portions of ether.

The extracted aqueous phase was made alkaline with dilute sodium hydroxide and extracted four times with 200 ml. portions of ether. This ether extract was dried over anhydrous sodium sulfate and reduced by flash evaporation to a 25-ml. volume. A 25-ml. volume of absolute alcohol was added and the phenethylamine precipitated as the hydrochloride through saturation of the solution with dry hydrogen chloride.

Phenethylbiguanide-C^{14}: β phenethylamine-C^{14} hydrochloride (3.5 gm.) and cyanoguanidine (1.7 gm.) were added to 1 ml. of water and the mixture heated at reflux for three hours. The solution was cooled to 110° C. and 8.7 ml. of 99 per cent isopropanol added with stirring. Agitation was continued for one hour and

† We are indebted to Dr. Seymour L. Shapiro of U. S. Vitamin & Pharmaceutical Corporation for his advice in this synthesis.

then the reaction mixture was set aside to crystallize overnight. The resulting solid was filtered and dried. M.P. 173-175° C. The crude product was recrystallized from methanol. M.P. 176-178° C.

Animal experiments: Twenty-four hour fasted male Sprague-Dawley rats were given phenethylbiguanide-C^{14} at the dosage level of 100 mg./kg. In one series of experiments the phenethylbiguanide-C^{14} was given orally and in the other the intraperitoneal route was employed. The animals were placed in metabolism cages to permit collection of the expired air, urine, and feces. One animal from each group was sacrificed at the end of 1, 2, 5, 8, 12, and 24 hours. The liver, kidneys, heart, lung, spleen, gastrointestinal tract with contents, testicles, epididymal adipose tissue, blood, and an aliquot of muscle from the femur were removed and rapidly frozen with dry ice and dehydrated by lyophilization. To permit more uniform planchetting of the tissue for the determining of its C^{14} content (direct counting), the fat and fat-like substances were removed from the dehydrated tissues with acetone and petroleum ether.

TABLE 1

Summary of per cent recovery of radioactivity following oral administration

	Hours after administration					
	1	2	5	8	12	24
Tissue						
Liver	16.2	13.6	2.4	1.9	1.0	1.9
GI tract and contents	71.4	65.6	47.8	33.1	29.3	16.2
Muscle*	2.1	3.6	0.4	0.9	0.0	0.0
All others†	0.9	0.5	0.2	0.1	0.1	0.1
Urine	7.6	16.5	39.2	—	68.9	97.2
Total	98.2	99.8	90.0	—	99.3	115.4

*Muscle value is calculated on the assumption that the muscle represents 50 per cent of total animal weight.

†Combined recovery of count in kidney, heart, lungs, spleen, testicles, and adipose tissue.

TABLE 2

Summary of per cent recovery of radioactivity after intraperitoneal administration

	Hours after administration					
	1	2	5	8	12	24
Tissue						
Liver	15.9	17.6	2.7	2.5	1.4	0.5
GI tract and contents	16.7	18.3	10.7	22.4	22.4	3.9
Muscle*	11.9	8.2	8.0	5.8	4.5	1.2
All others†	5.7	2.1	1.0	0.3	0.2	0.1
Urine	24.8	32.9	68.5	78.4	84.8	(134)‡
Total	75.0	79.1	90.9	109.4	113.3	139.7

*Muscle value is calculated on the assumption that the muscle represents 50 per cent of total animal weight.

†Combined recovery of count in kidney, heart, lungs, spleen, testicles, and adipose tissue.

‡The reason for this high value is not known.

Thus the C^{14} content was determined separately on the lipid and lipid-free residue. The data presented here for each tissue are the combined counts for these fractions.

RESULTS

No measurable amount of C^{14} was found in the expired air. The C^{14} label must therefore be quantitatively recovered in the excretion products and the body tissues.

A summary of the C^{14} recovered in the urine and tissues is given in tables 1 and 2. Since the total of the C^{14} recovered in the heart, kidneys, lungs, spleen, testicles, and epididymal adipose tissues was generally less than 5 per cent, the percentage recoveries for these tissues are combined in the tables. It is apparent that the C^{14} label is almost quantitatively excreted in the urine within twenty-four hours in both the orally and intraperitoneally administered animals.

In figures 1 and 2 are data showing the concentration of phenethylbiguanide-C^{14} per ml. of tissue water. The tissue water content was obtained by the loss in weight by dehydration. Two assumptions are made in these calculations. First, it is assumed that the C^{14} label was distributed uniformly in total tissue water, and second, that the C^{14} label represented unmetabolized phenethylbiguanide-C^{14}. Figures 1 and 2 demonstrate that the stomach plus GI tract and liver concentrated the unexcreted label almost to the exclusion of other tissues.

DISCUSSION

The high C^{14} content in the gastrointestinal tract of the intraperitoneally injected animals was of particular interest to us. In separate experiments the stomachs of three one-hour intraperitoneally injected animals were rinsed out with distilled water. In each case it was found that the chromatographically identified radioactive phenethylbiguanide was predominately in the juice rather than bound to the tissue. It thus appears that the drug is trapped by the acidic stomach juices.

Phenethylbiguanide with a pK_a of approximately 11[8] is probably one of the most alkaline drugs in use today. It is not surprising to find high concentrations of it in the stomach juices. This may speak well for the drug since its slow release from this reservoir would tend to prolong its duration of action. However, in view of the concept of Shore, Brodie, and Hogben[7] that passage across the stomach barrier is accomplished only by an unionized molecule, just how the certainly ionized phenethylbiguanide enters the stomach from the blood in the intraperitoneally injected rats will need further investigation.

It is apparent from the data in figures 1 and 2 that the

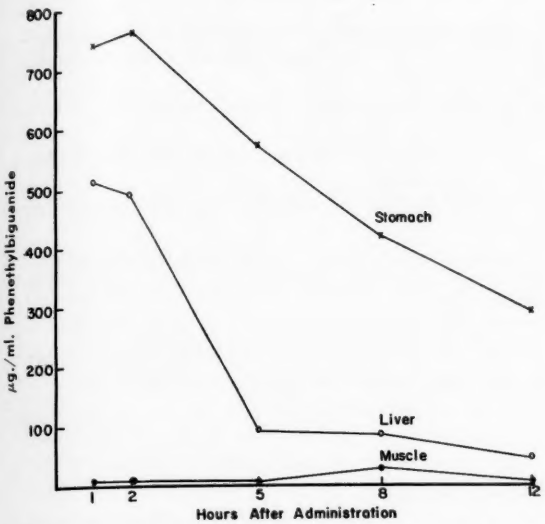

FIG. 1. Phenethylbiguanide (μg./ml.) in tissue water after oral administration. (Stomach refers to gastrointestinal tract and contents.)

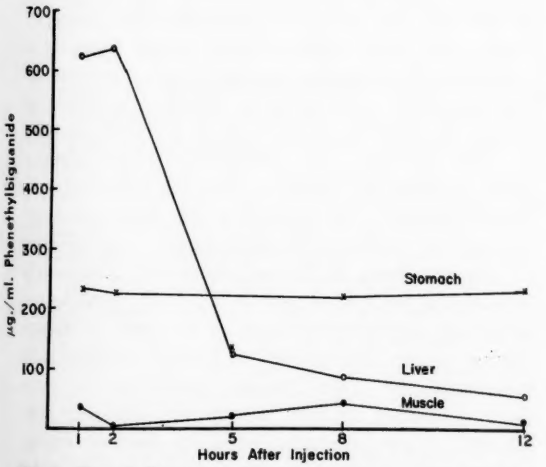

FIG. 2. Phenethylbiguanide (μg./ml.) in tissue water after intraperitoneal injection. (Stomach refers to gastrointestinal tract and contents.)

phenethylbiguanide-C^{14} concentration in the liver at one to two hours was sufficiently high to produce the same metabolic effect as observed under in vitro conditions[3] (i.e., 75 per cent inhibition of the oxidation of C_2 fragments by the Krebs cycle pathway employing adipose tissue). It should be pointed out that as a necessary consequence of following C^{14} distribution, phenethylbiguanide-C^{14} was administered at a dosage level of 100 mg./kg. This quantity is far in excess of that needed to produce a hypoglycemic effect in diabetic animals. It is likewise at least thirty times that normally employed as a human therapeutic dose (assuming a 200-mg. daily dose for a 60-kg. man). However, assuming the distribution of phenethylbiguanide-C^{14} to be the same at 1/30 of the dosage used in this study, sufficient quantities of the drug would still be present to induce the effect observed in in vitro studies at the lower end of phenethylbiguanide concentration gradient (i.e., 25 per cent inhibition of the oxidation of C_2 fragments by the Krebs cycle pathway).

A small inhibitory effect on the Krebs cycle pathway might still produce a major effect in increasing glycolysis in the liver by inhibiting the Pasteur effect. The increased glycolysis would lower sugar reserves and induce a hypoglycemic effect. Excess lactate or pyruvate formed would be conveyed to the muscle tissue to be oxidized by the Krebs cycle pathway there. Indeed the data of these experiments favor our previous suggestion[2] that muscle can carry out the oxidation of excess lactate-pyruvate produced in the liver, since in muscle tissue the phenethylbiguanide-C^{14} concentration was found to be very low.

We realize it is hazardous to draw too extensive a comparison between in vitro and in vivo observations at this time. Greater correlation between in vitro and in vivo effects, if indeed such a correlation exists, must await more detailed observation of the effects of the drug on intact animals, isolated tissues, and purified enzyme systems. Experiments are now in progress to identify the tissue and excretion products of phenethylbiguanide.

SUMMARY

Tissue distribution of the oral hypoglycemic agent, phenethylbiguanide, was determined by oral and intraperitoneal administration of the C^{14}-labeled drug to two separate groups of Sprague-Dawley rats at 100 mg./kg.

It was shown in both groups that the C^{14} label concentrated in the liver and gastric juice immediately following administration of the drug. After five hours approximately 30 per cent of the C^{14} was recovered in the urine and within twenty-four hours over 95 per cent of the C^{14} label was excreted.

SUMMARIO IN INTERLINGUA

Le Distribution Tissutal de Beta-Phenethylbiguanida Marcate con C^{14}

Le distribution in le tissus esseva determinate pro le oral agente hypoglycemic, phenethylbiguanida, post administrationes oral e intraperitoneal del droga marcate

per C^{14} a duo separate gruppos de rattos Sprague-Dawley in un dosage de 100 mg per kg de peso corporee.

Se monstrava in ambe gruppos que le C^{14} esseva concentrate in hepate e succo gastric immediatemente post le administration del droga. Post cinque horas, approximativemente 30 pro cento del C^{14} esseva retrovate in le urina, e intra vinti-quatro horas, plus que 95 pro cento del C^{14} esseva excernite.

ACKNOWLEDGMENT

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The precise etiology of obesity in man is unknown. The oft-repeated statement that obesity is caused by the ingestion of more calories than are expended in the same time period is factual but is a statement of the conditions which are required for depot lipid deposition rather than a statement of the cause of obesity. This is analogous to saying that the cause of edema is a positive salt and water balance, or that the cause of alcoholism is the excessive ingestion of alcohol. In all three situations, the true causes — and they are undoubtedly multiple — are to be found in the factors causing a disturbance in homeostasis within the organism. In the case of obesity, the problem relates to factors which cause an inappropriately positive caloric balance and eventually an inappropriately high body lipid content. In times of growth, convalescence, pregnancy and lactation, a positive caloric balance is physiologic and essential. In well-nourished adults, a positive caloric balance is unnecessary and undesirable.

The taking of food is, of course, a basic urge which arises within the central nervous system and is required for self-preservation. The homeostatic mechanisms which adjust the intake of food appropriately to the expenditure of energy are not known with certainty and probably involve both neurologic and biochemical pathways and signals. Glucostatic, lipostatic and thermostatic mechanisms have been suggested but none as of this date has been adequately validated in man.

The hypothalamus appears to be the coordinating center for both afferent and efferent impulses which traverse the basic reflex arc adjusting food intake to tissue needs. This reflex may be looked upon as a servo mechanism although the signals which account for both the short and long range control of appetite in relation to exertion and changes in body composition are still unknown. The cerebral cortex undoubtedly exerts an important influence upon this reflex in man. Neural pathways exist from the frontal lobes and other parts of the cortex to the hypothalamus and provide the anatomic basis for the well-established relationship between the psyche and appetite. Certainly psychogenic factors are of great importance in the onset of human obesity. In our own studies we have found that such meaningful and often traumatic experiences as marriage, pregnancy, surgery, menopause, death or separation of a loved one may herald the onset of obesity. Under these conditions "conflicts" arising in the frontal lobes could generate signals which might modify the activity of the satiety center in the ventromedian nucleus of the hypothalamus and lead to relative hyperphagia. Further research is needed to clarify the homeostatic mechanism which operates in the healthy individual so that a more rational basis will become available for pinpointing defects which occur in disease.

From "Obesity as a Nutritional Disorder," by Robert E. Olson, in *Federation Proceedings*, Volume 18, Number 2, Part II, pp. 60-61, July 1959.

The Effects of Phenformin on the Isolated Rat Diaphragm

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Many of the effects of DBI, added to tissues in vitro, are now well documented. It can cause a marked increase in both the glucose uptake and the glycogen breakdown of the diaphragm[1] and in lactate production by this tissue.[2] Oxygen utilization of whole tissues and of homogenates is reduced.[3,4] We have repeated and extended some of these observations, and in addition, we have measured phosphate output by the isolated rat diaphragm under the influence of DBI. In an effort to gain more information on the mechanism of action of DBI, we have also measured the influence of this drug on the volume of distribution of certain pentoses. The results to be described give only a portion of our experimental data. More complete details will be reported elsewhere.

EXPERIMENTAL

Normal (or alloxan diabetic) rats weighing 200 to 250 gm. were sacrificed by stunning. The diaphragm was then quickly removed and was trimmed and cut into two more or less equal portions—the hemidiaphragms. A small portion of each hemidiaphragm was cut off for a determination of the initial glycogen level.[5] The remaining two pieces were lightly blotted, weighed and added to the appropriate incubation medium. A modified Krebs-Henseleit medium was used, with the phosphate salt omitted. Five milliliters of the medium were placed in 25-ml. flasks, which were gassed with 95 per cent O_2-5 per cent CO_2 mixture after the tissues were added. In some of the flasks, DBI had been added to give a final concentration of 1.0 mg. per ml. Glucose or fructose was present in a concentration of 200 mg. per 100 ml. Glucose was determined by the Somogyi-Nelson method[6] or by a fermentation method, using glucose oxidase (Sigma). Inorganic phosphate was determined by the method of Fiske and SubbaRow,[7] lactate by the method of Barker and Summerson.[8]

In the experiments in which pentose spaces were measured, the intact diaphragm preparation of Kipnis and Cori[9] was used. Tissues were incubated for one hour at 37° C. in a Krebs-Henseleit medium which contained 400 mg. per cent pentose. Following the incubation, the diaphragm was removed and trimmed and a small portion was used for a determination of the water content. The remaining tissue was weighed, placed in boiling water and extracted for ten minutes. Aliquots of the resulting solution were used for the required analytical determinations. Pentoses were determined by the method of Roe.[10]

RESULTS

Table 1 shows the results of experiments in which glucose or fructose uptakes were measured, along with changes in glycogen levels (initial glycogen level less

TABLE 1

Effect of DBI in vitro

Animal	Normal		Diabetic		Normal	
Monosaccharide in medium	Glucose		Glucose		Fructose	
Monosaccharide uptake (mg./gm.)		N		N		N
Control	3.60	10	3.64	5	1.58	4
DBI	4.70	10	6.17	5	2.50	4
Diff.	1.10		2.53		0.92	
P	<0.01		>0.05		<0.01	
Glycogen change (mg./gm.)						
Control	0.52	6	1.20	4	—1.30	4
DBI	—1.00	6	—0.82	4	—1.85	4
Diff.	—1.52		—2.02		—0.55	
P	<0.01		<0.01		<0.01	
Lactate output (mg./gm.)						
Control	1.83	6	2.78	4	—	
DBI	3.49*	6	3.22	4	—	
Diff.	1.66		0.44		—	
P	<0.05		>0.05		—	
Phosphate output (mg./gm.)						
Control	0.55	6	0.45	3	0.44	4
DBI	0.63	6	0.73	5	0.65	4
Diff.	0.08		0.28		0.19	
P	>0.05		<0.025		>0.05	

*DBI conc. 0.5 mg./ml. for this particular experiment. In all other experiments the DBI concentration was 1 mg./ml.
N = Number of experiments.

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glycogen level after incubation). Values for lactate output and phosphate output are also given. DBI exerted a marked stimulating effect upon glucose and fructose uptake in tissues from normal animals; it had a similar effect on glucose uptake on tissues from diabetic animals. Glycogen breakdown was greatly increased, and there was an increase in lactic acid production. The increase in phosphate liberation was not quite so pronounced, but is nevertheless significant. Under similar experimental conditions, insulin decreased phosphate loss to the medium. The source of the phosphate may be rather important. It must be either phosphate esters such as the C_6 or C_3 compounds, or it may come from compounds with a high energy bond. If the source is one of the esters of the Embden-Meyerhof chain, then it seems likely that the production of lactic acid would be interfered with, and there would be a reduced lactate output, rather than an increased output. No direct measurements of ATP, ADP or CP were made, but these compounds would seem to be likely sources of the phosphate.

The results of an experiment similar to the well-known one of Park et al.[11] in which the tissues are incubated in a medium containing an extremely high concentration of glucose are shown in table 2. The figures suggest that insulin increases the intracellular glucose, but the increase is not statistically significant. However, since the experiment was essentially a repetition of the work of Park,[11] who found a significant increase in intracellular glucose, the action of insulin in these experiments appeared to be confirmed. DBI, on the other hand, caused a drop in intracellular glucose. This gives further confirmation to the idea that DBI increases the rate of glucose breakdown.

The effect of DBI, and of some other substances upon the value of distribution of some different pentoses is shown in table 3. It can be seen that DBI, in contrast to insulin or dinitrophenol, does not increase the pentose space. The water content and the insulin space of the diaphragm are unaltered by the drug (table 4). The failure of DBI to affect these values shows that any increase in sugar uptake (on the assumption that glucose and these pentoses are transported by a similar mechanism) cannot be ascribed simply to an increase in extracellular or intracellular fluid. Its effect must therefore be on one of the metabolic processes of the cell. Other workers have shown that it interferes with oxidative processes,[3,4] and it has been amply demonstrated by Randle[12] and others that anaerobiosis, or the presence of an inhibitor of aerobic metabolism may, in suitable media, stimulate glucose uptake or glycogen breakdown. The mode of action of DBI may be related to this.

TABLE 2

Effect of DBI on free glucose content of diaphragm*

Control	Insulin	N	
5.81 ±0.31	6.21 ±0.03	3	No significant difference on small number of experiments.
Control	**DBI**		
6.22 ±0.50	5.20 ±0.48	7	P 0.025

*Diaphragms incubated in heparinized dog plasma with glucose added to give a final concentration of 2,000 mg. per cent.

All values expressed as mg./gm. tissue, means ± standard deviation.

TABLE 3

Volume of distribution of pentoses (as per cent of wet weight, means ± standard deviation)

	D-xylose	N	L-arabinose	N	D-arabinose	N	D-xylose (phosphate buffer)	N
Control	53.7 ±3.5	5	42.3 ±1.5	4	40.8 ±5.0	4	46.4 ±2.6	5
DBI	53.4 ±1.8	5	48.4 ±4.3	4	—		45.0 ±7.8	4
Insulin	74.3 ±1.5	4	—		66.0 ±8.1	4	76.9 ±3.0	4
Dinitrophenol (2.5×10^{-3}m)	69.5 ±4.9	7	—		—		66.4 ±4.9	5

N = Number of experiments.

TABLE 4

Effect of DBI on insulin space of water content

	Insulin space (per cent wet weight)	Water content (per cent wet weight)	N
Control	22.5 ±1.4	76.5 ± .7	8
DBI	24.8 ±3.1	76.5 ±0.7	8
Insulin	22.5 ±0.8	75.5 ±0.9	4
Dinitrophenol (2.5×10^{-5}m)	22.8 ±2.7	80.5 ±0.6	8

N = Number of experiments. All values are mean values, ± standard deviation.

The effect of DBI again raises the question of the fundamental mechanisms which influence glucose uptake of the cell. Certainly permeability and glucose uptake are related, but the fact that conditions can be obtained, as with DBI, whereby there is an increased glucose uptake without the attendant increase in permeability, strongly suggests that this glucose uptake is influenced by many factors, of which the permeability of the cell is only one.

SUMMARY

Phenformin in vitro causes an increase in glucose uptake, glycogen breakdown, lactate output and output of inorganic phosphate of the isolated rat diaphragm.

Phenformin does not cause an increase in pentose space, insulin space, or water content of the intact isolated diaphragm.

SUMMARIO IN INTERLINGUA

Le Effectos de Phenformina Super le Metabolismo del Isolate Diaphragma del Ratto

In le isolate diaphragma del ratto, phenformin causa in vitro un augmento del fixation de glucosa, del decomposition de glycogeno, del rendimento de lactato, e del rendimento de phosphato inorganic.

In le intacte isolate diaphragma del ratto, phenformin non causa un augmento del spatio de pentosa, del spatio de insulina, o del contento de aqua.

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Developmental Genetics

A step up in the biological hierarchy is the problem of development. There is not much of a nonspeculative character that can be said of histogenesis, which rests on the still enigmatic relations of genes and cytoplasm to which we have referred. With respect to morphogenesis there is at least increasing contact between geneticists and embryologists. There was a period not long ago when experimental embryologists would trace the interactions of various factors in the development of an organ—pressures and tensions, inductions, hormones, neural stimulation, environmental conditions—and perhaps, at the end, list heredity as a sort of magic that operated through other than physiological channels. More recently, many trained embryologists have turned to genetically determined abnormalities as useful material, with illuminating results. The systematic study of the interaction effect of numerous loci on particular characters is especially promising.

The complete analysis of the development of a higher organism nevertheless remains one of the most intractable problems of science. Perhaps it is beyond human grasp. But I suspect that there will be great advances in understanding and am sure that this can come about only in the conjunction of the two disciplines.

Beyond morphogenesis is the genetics of behavior on which a beginning has been made.

From "Genetics and the Hierarchy of Biological Sciences," by Sewall Wright, in *Science*, Vol. 130, No. 3381, pp. 959-65, Oct. 16, 1959.

The Mechanism of Action of Hypoglycemic Guanidine Derivatives

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Renewed interest in guanidine derivatives as possible oral therapeutic agents in the treatment of diabetes has followed the discovery by Ungar et al.[1] that certain biguanides are hypoglycemic agents of low toxicity. This report is concerned with the mechanism of action of this new group of compounds as exemplified by one of them, phenethylbiguanide (DBI), as compared with that of other guanidine derivatives. As long ago as 1918, Watanabe[2] showed that guanidine elicited a pronounced hypoglycemic response in rabbits. However, the toxic manifestations of this substance precluded its trial as a possible therapeutic agent in diabetes mellitus. An intensive search by Frank et al.[3] led to the synthesis of decamethylenediguanidine (Synthalin) which was found to exhibit enhanced hypoglycemic activity associated with markedly diminished toxicity. However, after intensive clinical trial the therapeutic use of Synthalin as an oral hypoglycemic agent was abandoned. Disagreeable side effects including nausea and weakness were frequent. Furthermore, except for lowering blood sugar and diminishing glycosuria, Synthalin therapy did not really correct the aberrant metabolism of the diabetic individual. Elevations in blood and urinary lactate, citrate and other organic acids were observed.[4] In a balance study of diabetics undergoing Synthalin therapy, Kaufmann-Cosla and Vasilco found that urinary glucose was supplanted by large excretions of other organic compounds.[5]

In vitro studies on the effect of phenethylbiguanide (DBI) on rat diaphragm by Williams et al.[6] have demonstrated increased glucose uptake, increased lactate production and increased glycogenolysis. These results are indicative of the action of a respiratory inhibitor. Randle and Smith[7] have demonstrated increased glucose uptake by isolated rat diaphragm subjected to oxygen lack as well as a number of cytotoxic substances including dinitrophenol, cyanide and arsenite. Steiner and Williams[8] have studied the effect of Synthalin and phenethylbiguanide on subcellular preparations from various tissues and conclude that a major site of inhibition of cellular respiration involves cytochrome oxidase. Wick and Larsen,[9] on the other hand, report that cytochrome oxidase is not inhibited, but rather some point prior to cytochrome c in the succinic oxidase system. These authors, working with rat epididymal adipose tissue, also observed that the degree of inhibition of oxidation of several Krebs cycle intermediates by phenethylbiguanide differed considerably: 48 per cent for succinate, 16 per cent for fumarate and only 3 per cent for citrate. In our own experience with rat kidney homogenates we observed a 58 per cent inhibition of fumarate oxidation and a 49 per cent inhibition of succinate oxidation with 2×10^{-3} M phenethylbiguanide.

Hollunger[10] has recently advanced another view concerning the nature of the inhibition of oxygen uptake by guanidine and its derivatives. Working with rabbit kidney mitochondria and rat liver mitochondria, he observed that when phosphorylation became uncoupled from oxidation either by aging, treatment with calcium ions, or by the addition of an uncoupling agent such as 2,4-dinitrophenol (DNP), guanidine no longer inhibited oxygen uptake. Partial restoration of the phosphorylative capacity of the aged or calcium treated mitochondria by addition of diphosphopyridine nucleotide (DPN), adenosine triphosphate (ATP) and manganous ions was accompanied by partial return of susceptibility to guanidine inhibition. Figure 1 illustrates the mechanism postulated by Hollunger to explain these phenomena. Normally, electron transport via the pyridine nucleotide-flavoprotein-cytochrome chain is coupled to the generation of high-energy bonds which eventually

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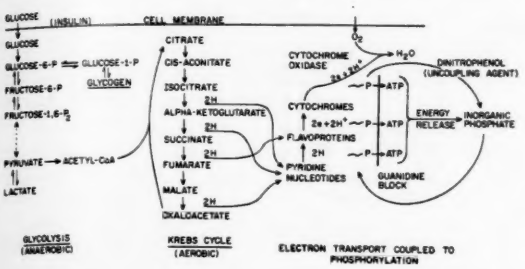

FIG. 1. Scheme showing postulated site of action of guanidine derivatives.

interact with adenosine diphosphate (ADP) to form ATP. These primary high energy bonds must be removed before the respiratory chain can resume electron transport; any block to their removal, in effect, blocks respiration. According to Hollunger, guanidine and its derivatives interfere with the interaction of ADP with the high-energy bond containing chain. Uncoupling agents such as DNP bypass this block by causing wasteful release of respiratory chain high-energy bonds, and, indirectly, release the inhibition of electron transport. Aged or calcium treated mitochondria either do not couple at all during oxidation, or the high-energy bonds formed are unprotected from spontaneous hydrolytic processes; in either case guanidine would not be expected to inhibit oxygen uptake.

To test this hypothesis as a possible mechanism of action of phenethylbiguanide inhibition of oxygen uptake, rat kidney mitochondria were prepared from isotonic sucrose homogenates by differential centrifugation at 0° C., and after several repeated washings and centrifugations, dispersed in a known volume of isotonic sucrose. Figure 2 shows the results of a typical manometric run employing fumarate as a substrate. Each point represents the average ten-minute oxygen uptake of two simultaneously run experiments with the same mitochondrial preparation. Initially there is little difference between the uptake in the control flasks and those with added DNP. However, with time the respiratory rate of the latter gradually declines. The flasks with phenethylbiguanide as the sole addition exhibit a distinctly lower uptake than the controls from the start. Those with phenethylbiguanide plus DNP both added at the beginning show a distinct recovery of respiratory activity during the initial period, but this gradually falls off as do those with DNP alone. The remaining curve shows the effect of DNP addition after twenty minutes upon a phenethylbiguanide inhibited system. There is marked rise in oxygen uptake during the next two ten-minute periods, followed by a gradual decline, generally following the system with DNP alone. Figure 3 shows the same data in terms of accumulated oxygen uptake during the same period of time.

These results are consistent with the mechanism proposed by Hollunger, and are difficult to explain in terms of cytochrome oxidase or succinic oxidase inhibition per se. It is postulated that the in vitro metabolic effects of phenethylbiguanide can be best explained by a primary interference with the transfer of respiratory chain high-energy bonds to ADP, secondarily blocking electron transport, which is reflected in diminution of oxygen uptake. This results in the creation of a partially anaerobic state which diminishes the Pasteur effect which normally limits glycolysis in respiring cells. Glucose uptake and conversion to lactate are therefore increased. At the same time since oxidation is reduced, certain Krebs cycle intermediates such as citrate might be expected to accumulate. This mechanism is consistent with the reported metabolic actions of phenethylbiguanide in humans, although the possibility of differential tissue localization may complicate the over-all in vivo metabolic response.

SUMMARY

1. Phenethylbiguanide inhibits oxygen uptake.

2. Dinitrophenol releases this inhibition.

3. These results are consistent with Hollunger's concept that guanidines inhibit transfer of high-energy bonds to ADP, thereby secondarily inhibiting oxygen uptake.

4. The metabolic effects of phenethylbiguanide, i.e., hypoglycemia, diminished tissue glycogen, and accumulation of citrate, pyruvate and lactate can be interpreted by this mechanism.

SUMMARIO IN INTERLINGUA

Le Mechanismo del Effecto Hypoglycemic de Derivatos de Guanidina

1. Phenethylbiguanida inhibi le acceptation de oxygeno.

2. Dinitrophenol relaxa iste inhibition.

3. Iste resultatos es compatibile con le conception de Hollunger que derivatos guanidinic inhibi le transferimento de ligamines de alte energia a adenosinediphosphato, inhibiente assi secundarimente le acceptation de oxygeno.

4. Iste mechanismo es capace a interpretar le effectos metabolic de phenethylbiguanida, i.e. le hypoglycemia causate per illo, le reducite glycogeno tissutal, e le accumulation de citrato, pyruvato, e lactato.

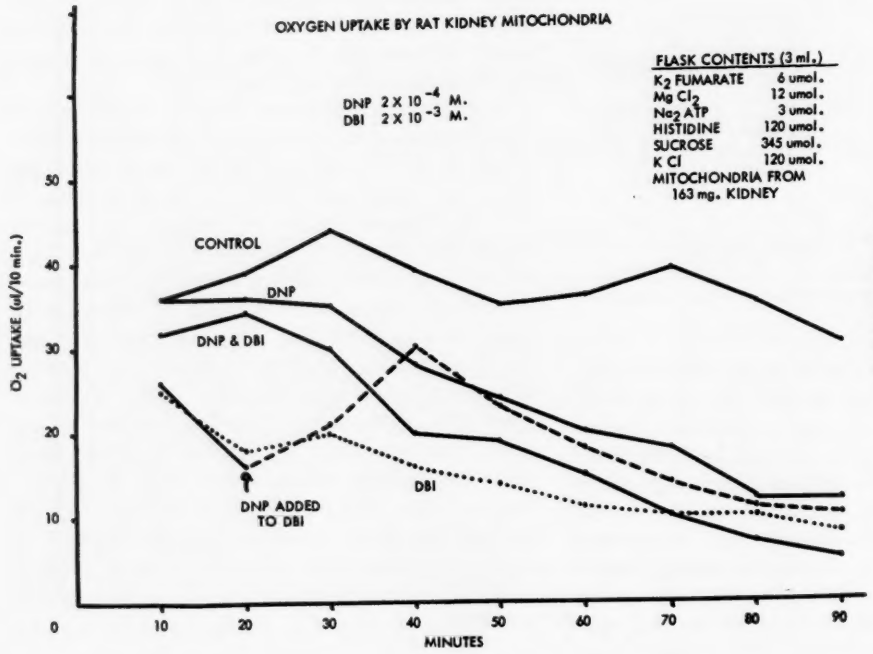

FIG. 2. Effect of phenethylbiguanide (DBI) and dinitrophenol (DNP) on respiration of rat kidney mitochondria utilizing fumarate as substrate. Successive ten-minute increments of oxygen shown on ordinate.

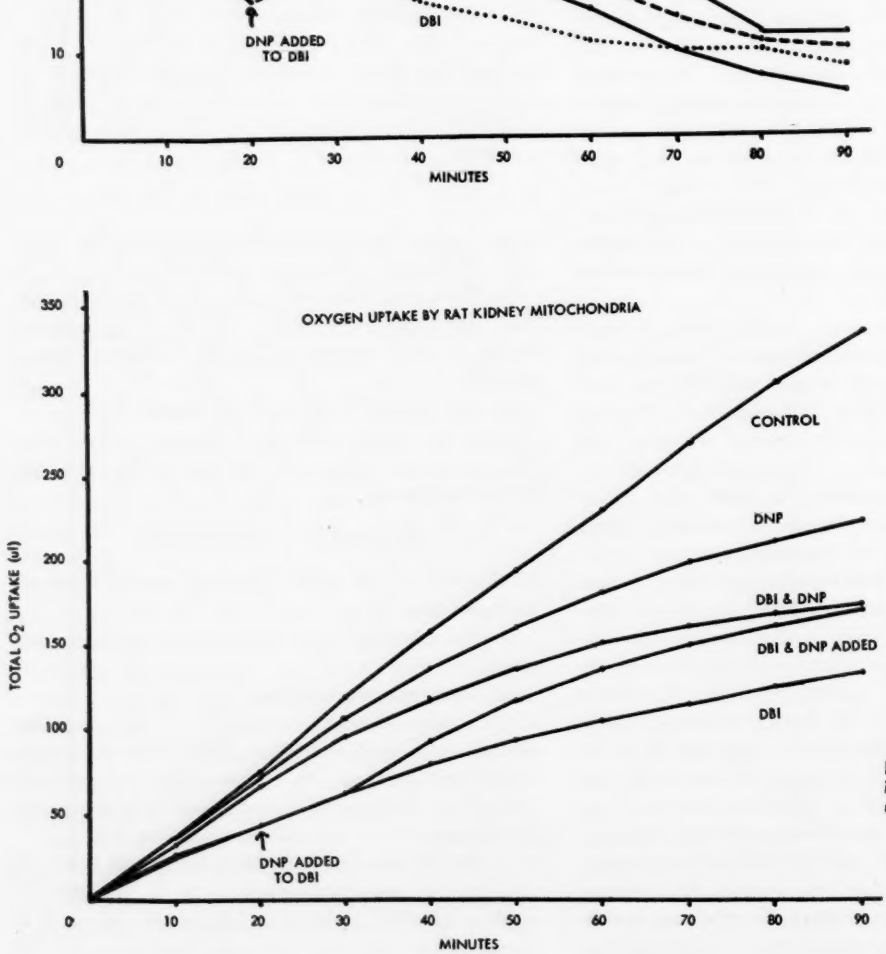

FIG. 3. Same system as in figure 2. Total oxygen uptake shown on ordinate.

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That unesterified fatty acids appear very rapidly in the blood during chylomicron metabolism had been shown by R. J. Havel and D. S. Fredrickson (*J. Clin. Invest.* 35:1025, 1926). They used isotopically labeled chylomicrons which were secured from dogs in which a cannula had been placed in the thoracic lymph duct. Each dog was given 3.6 gm. to 10.8 gm. of fat in the form of cream by stomach tube. The cream contained sodium palmitate-1-C^{14}. The chylomicrons were separated from the lymph, washed, and resuspended in 0.5 M sodium chloride solution. The resulting emulsion was given by intravenous injection to two adult female dogs. These dogs had been fasted for twenty hours prior to the injection. During the injection, the dogs were maintained under light ether anesthesia. Physiologic saline was given by vein during the infusion of the chylomicrons and the collection of subsequent blood samples.

The chylomicrons disappeared exponentially from the blood with a half life of fifteen to twenty-four minutes. The triglyceride and phospholipid fractions of the chylomicrons disappeared at the same rate. The specific activity of the plasma unesterified fatty acids rose during the clearing of the plasma and reached a level exceeding 50 per cent of the specific activity of the transfused triglyceride fatty acids. The work of Havel and Fredrickson showed that the half life of the esterified fatty acid fraction was about two minutes.

Havel and Fredrickson suggested that there is an intimate relationship between the removal of chylomicrons and the hydrolysis of their constituent triglycerides. The site where this hydrolysis of the triglycerides occurs is unknown. Regardless of the tissue responsible for the removal of the chylomicrons from the blood, part of the unesterified fatty acids released during this process is returned rapidly to the blood stream. Here the fatty acids combine with serum albumin and are transported as a protein-fatty acid complex.

The above work indicates that although the unesterified fatty acids form a small fraction of the total blood lipids, their rate of turnover suggests that they play a very important role, especially in the transport of lipids from the adipose tissue to such organs as the liver. The liver appears able to remove these fatty acids from the blood. The rate of removal of the unesterified fatty acids by the liver appears to occur even when no other lipid fractions are being removed from the blood.

With the increasing activity in this field of lipid metabolism, it is likely that additional evidence will soon be forthcoming to delineate more clearly the exact role of unesterified fatty acids in metabolic reactions.

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Histologic and Histochemical Observations in the Rabbit After Phenethylbiguanide

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Ungar and his co-workers[1] recently reported that phenethylformamidinyliminourea hydrochloride, referred to as DBI, administered orally or parenterally caused significant hypoglycemia in various animal species. Subsequent studies by Pomeranze et al.,[2] and Krall and Camerini-Davalos[3] demonstrated that this drug lowers the blood sugar in a large number of diabetic patients.

Any substance lowering the blood sugar may act either by increasing insulin output from the pancreas, by inhibiting glucose output from the liver or through increase in peripheral uptake of glucose in skeletal muscle or in fat. Although not directly involved in carbohydrate metabolism the kidney, through glomerular filtration and tubular reabsorption of glucose, contributes—albeit indirectly—to maintenance of blood sugar homeostasis.

Various authors have attempted to elucidate the mode of action of DBI. It was thus noted that it was effective in the alloxanized animal[4] as well as in the depancreatized and in the hepatectomized animal.[4] Williams and associates[5,6] concluded from in vivo and in vitro studies in guinea pigs that it lowers the blood sugar by promoting anaerobic glycolysis in muscle with increased glucose utilization and by causing decreased gluconeogenesis from proteins with a reduction in the output of glucose from the liver. More recently Wick and associates[7] demonstrated by in vitro studies using rat adipose tissue that DBI may exert a metabolic block at one of the events of the Krebs cycle in the conversion of succinate to fumarate.

The present study was undertaken to determine whether DBI has any effect on histochemically demonstrable glucose-6-phosphatase, succinic dehydrogenase as well as acid and alkaline phosphatase, esterase, adenosine triphosphatase and 5-nucleotidase in the various tissues involved in blood sugar homeostasis. Furthermore, an attempt was made, using histologic technics and special stains, to determine whether these drugs caused any pathologic alterations in these tissues.

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MATERIAL AND METHODS

One hundred and seventy rabbits of both sexes, weighing between 2,500 and 3,000 gm., were divided into three groups: Group A, consisting of thirty rabbits, was given 25, 50 or 100 mg./kg. of DBI intravenously and sacrificed five hours later. Group B included forty rabbits of which twenty received a single dose of 100 mg./kg. of DBI in the morning only, while the other twenty were given one injection of DBI in the morning and one in the evening, each injection again 100 mg./kg. All animals were sacrificed twenty-four hours after the initial injection. Group C consisting of 100 animals received 25, 50 or 100 mg./kg. of DBI intravenously once or twice daily for periods up to three months. Twelve normal rabbits served as controls.

During the acute experiments the blood sugar levels were determined hourly for three to four hours. During long-term administration of the drug daily fasting blood sugar determinations were carried out. The blood sugar levels were determined by the Nelson-Somogyi method.[8]

All rabbits were kept in metabolism cages and received Purina rabbit chow and water ad libitum. All animals were sacrificed by overdosage with sodium Nembutal and the pancreas, liver, kidney and skeletal muscle were removed immediately after death and fixed in Zenker formol solution. The blocks were embedded in paraffin and all tissues were stained with hematoxylin and eosin stain. The pancreas and liver, in addition, were stained by the PAS trichrome technic controlled by diastase digestion,[9] by Gomori's aldehyde fuchsin stain[10] and by the chrome alum hematoxylin phloxine technic.[11]

For the enzymatic staining reactions sections were cut from thin tissue blocks that had been fixed for eighteen hours in cold 6 per cent neutral calcium formol or from quick frozen tissue utilizing the cryo-

stat. The following staining technics were utilized:

Glucose-6-phosphatase: The procedure used was a modification of that originally described by Chiquoine[10] and subsequently modified by Wachstein and Meisel.[12]

Succinic dehydrogenase: The technic of Seligman and Rutenburg[14] was used on cryostat sections. The indicator substance was neotetrazolium chloride. Incubation time was thirty to 120 minutes.

Esterase: The azo-dye method using alpha-naphthyl-acetate was employed for visualization of esterase activity in fresh frozen as well as in formalin-fixed sections.[15]

Nonspecific alkaline phosphatase: Sections of both fresh and neutral formalin-fixed tissues were stained according to Gomori's lead nitrate technic using ammonium sulfide for the demonstration of the formed lead phosphate precipitate. In addition, the azo-dye method using alpha-naphthyl-phosphate was employed.[16]

Nonspecific acid phosphatase: Gomori's lead phosphate technic was employed on unfixed frozen sections utilizing glycero-phosphate as the substrate.[16]

5-nucleotidase: Fresh frozen sections were incubated from five to sixty minutes using the modification of Gomori's lead nitrate technic[16] described by Wachstein.[17]

Adenosine triphosphatase: The technic employed was similar to that employed for 5-nucleotidase with the exception that the muscle adenylic acid was replaced by an equivalent amount of adenosine triphosphate.[19]

RESULTS

In the short-term experiments a decline of the blood sugar of up to 60 mg. per cent was observed within three to four hours. During long-term administration of DBI the daily fasting blood sugar levels usually were within normal levels. Histologic examination of liver, pancreas and skeletal muscle showed no morphologic alterations. Similarly no renal changes were observed except in two rabbits who received 100 mg./kg. of DBI twice daily for six consecutive days. In these animals hyaline droplets (figure 1) and vacuolization (figure 2) were found in the epithelial cells of the distal convoluted tubules of the kidney. These vacuoles contained neither fat nor glycogen. Moreover, histochemical studies showed also a reduction of succinic dehydrogenase in the kidneys of these two rabbits. This dose proved to be rather toxic and only four out of twelve animals of this group survived for this length of time. No other morphologic changes were observed at this dosage level in the other organs studied. In none of the rabbits did histochemical studies show any alterations in glycogen content of the liver and pancreas before or after DBI administration. Similarly, no changes of enzyme activity, except for those described above in the kidneys, were observed in the various other organs after long-term administration of the drug as compared with the normal, even when given in toxic dosages.

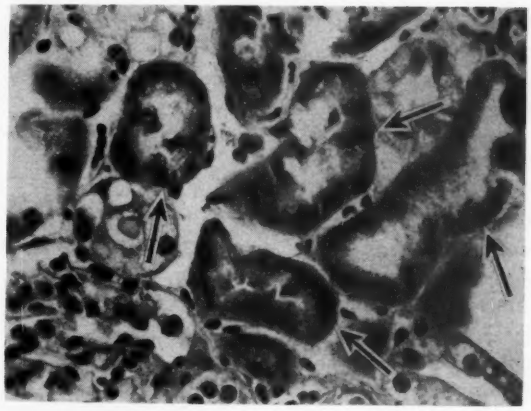

FIG. 1. Hyaline droplets (arrows) in renal epithelial cells of convoluted tubules of a rabbit which received 100 mg./kg. of DBI twice daily for six days. Hematoxylin and eosin, × 396.

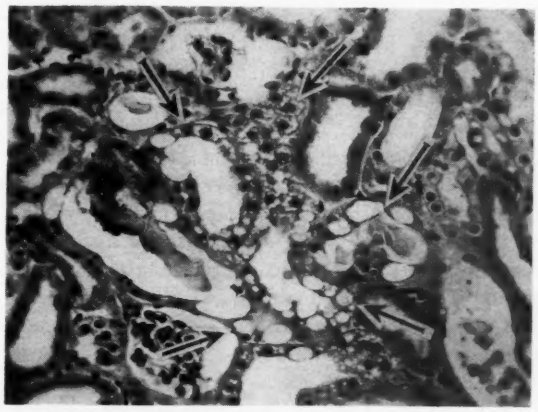

FIG. 2. Vacuolization (arrows) of renal epithelial cells of convoluted tubules of a rabbit after 100 mg./kg. of DBI twice daily for six days. Hematoxylin and eosin, × 198.

DISCUSSION

The present study indicates that DBI does not cause histologic or histochemical alterations in pancreas, liver or skeletal muscle. Similarly, no renal changes were observed after administration of moderate dosages of the drug.

In two animals receiving large amounts of the drug, i.e., 100 mg./kg. twice daily for six consecutive days there was hyaline droplet formation as well as

vacuolization of the distal convoluted tubules of the kidney. These changes are probably indicative of toxic cellular damage and have no relationship to the hypoglycemic action of the biguanide.

Recently, Creutzfeldt and Moench[19] reported that DBI administered to guinea pigs for up to four days caused degranulation and vacuolization of the pancreatic A cells. They, furthermore, observed hepatic necrosis as well as changes in the kidneys which consisted of fatty degeneration and necrosis of tubular epithelium. The findings in pancreas and liver are at variance with those in the present study. It is probable that species differences account for these discrepant observations.[19]

There are certain histochemically demonstrable intracellular enzymes which may serve as indicators for cell metabolic activity in relation to glucose utilization. Succinic dehydrogenase is known to participate in the Krebs cycle in the transformation of succinate to fumarate. The distribution of this enzyme in the liver was the same both before and after DBI administration. In general, no alterations could be detected within the kidneys except in two rabbits treated with 100 mg./kg. of DBI twice daily for six days. In both these animals a marked reduction of deyhdrogenase activity was found. This observation seems to be in keeping with the recent report of Wick and associates[7] who demonstrated in in vitro studies of fat that DBI exerts a blocking action at one of the events concerned with the conversion of succinate to fumarate and localized the most sensitive point of action as between succinic dehydrogenase and reduced cytochrome C. This action of DBI was stated to increase glycolysis and thereby to provide greater glucose utilization and consequent lowering of the blood sugar level. However, it seems unlikely that an inhibition of succinic dehydrogenase activity should increase glucose utilization. Furthermore, the action of DBI is probably a toxic effect of overdosage and not related directly to its hypoglycemic action.

Glucose-6-phosphatase is believed to be important for maintenance of blood sugar by contributing glucose from glucose phosphate in liver and kidney. Presumably an inhibition of this enzyme might be responsible for the hypoglycemic action of DBI. However, histochemical studies revealed no alterations in its activity in liver or in kidney even with toxic doses of the drug. Similar results have been obtained with biochemical studies in liver.[5]

Adenosine triphosphatase and 5-nucleotidase are believed to function in transport across the cell membrane.[20] Although the roles of acid and alkaline phosphatase and of esterase have not been clearly defined, they presumably may be used as indicators of metabolic activity and of the functional integrity of cells. In our studies none of these five enzymes showed any difference of activity after DBI as compared to the control animals.

The present findings do not shed additional light on the mechanism of action of DBI. They do, however, indicate that in the rabbit moderate dosages of DBI do not produce morphologically demonstrable alterations suggestive of toxicity. On the other hand after large doses, renal damage was observed in two instances. Furthermore, the drug does not alter the activity of the various enzymes studied.

SUMMARY

A study has been conducted on the effect of phenformin administration on rabbit pancreas, liver, kidney and muscle. There were no histological alterations in pancreas, liver or muscle attributable to the phenformin. Similarly there were no changes in histochemically demonstrable glucose-6-phosphatase, succinic dehydrogenase, alkaline or acid phosphatase, esterase, adenosine-triphosphatase or 5-nucleotidase even after long-term administration. However, in two animals given large amounts of phenformin for six days hyaline droplet formation and vacuolization of the distal convoluted tubules as well as a reduction in succinic dehydrogenase in the kidneys was found. These findings suggest a toxic effect due to overdosage of the drug. On the other hand, relatively small doses of phenformin do not produce morphologically demonstrable alterations of the kidney suggestive of toxicity.

SUMMARIO IN INTERLINGUA

Observationes Histologic e Histochimic in Conilios Tractate con Phenformin

Esseva studiate le effecto del administration de DBI in le pancreas, le hepate, le ren, e le musculos del conilio. Esseva trovate nulle alterationes attribuibile a DBI in pancreas, hepate, o musculo. Similemente, post cursos perdurative nulle alterationes esseva trovate in histochimicamente demonstrabile glucosa-6-phosphatase, dishydrogenase succinic, phosphatase alcalin o acide, esterase, adenosino-triphosphatase, o 5-nucleotidase. Tamen, in duo animales que recipeva quantitates excessive de DBI durante sex dies, un formation de guttettas hyalin e vacuolisation del convolute tubulos proximal si ben como un reduction del dishydrogenase succinic in le renes esseva constatate. Iste constatationes suggere le occurrentia de un effecto toxic in consequentia de hyperdosation del droga. Del altere latere, relativemente

micre doses de DBI non produce morphologicamente demonstabile alterationes in le renes de character suggerente le occurrentia de toxicitate.

ACKNOWLEDGMENT

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To determine whether comparable differences in water accumulation in the stomach occurred, rats that had been trained to eat 5 gm. of food in thirty minutes were killed at intervals after eating. The per cent of moisture and the dry weight of the stomach contents were determined. Up to seven hours after the feeding period the sucrose-fed rats had considerably more material in their stomachs than the dextrin-fed rats. The stomach content of the sucrose-fed rats was 1.2 times as great as that of the dextrin-fed rats one hour after feeding; the difference increased with time until the fifth hour, when the stomach content of the sucrose-fed rats was two times that of the dextrin-fed rats. The difference in the size of the stomach contents was attributable almost entirely to water. The stomachs of the sucrose-fed rats had from 2 to 4 ml. more water in them than those of the dextrin-fed rats for the first five to seven hours after feeding. In spite of the differences in stomach contents, there was no essential difference in the rate at which solids left the stomachs of the two groups of rats.

This observation suggested to the investigators that "the type of carbohydrate consumed can influence stomach volume appreciably through its effect on the amount of water retained in the stomach. Stomach volume increased, whereas food intake and growth decreased, when a carbohydrate of low molecular weight was substituted in the diet for one of high molecular weight." Harper and Spivey postulated that the reason rats grow equally well on a high protein diet, regardless of the type of carbohydrate, is that "the replacement of some of the low molecular weight carbohydrate by protein which exerts less osmotic pressure" permits the rat to eat more and "that less of the more adequate diet is required to satisfy the amino acid requirements of the rat."

From *Nutrition Reviews*, Vol. 17, No. 4, pp. 108, 109, April 1959.

Special Article

Laboratory Studies with Phenformin

Panel Discussion

Arne N. Wick, Ph.D., San Diego, Moderator

Robert Bolinger, M.D.
Kansas City, Kansas

Seymour Shapiro, Ph.D.
New York City

Donald W. Clarke, Ph.D.
Toronto

Georges Ungar, M.D., Sc.D.
New York City

Fred A. Kruger, M.D.
Columbus, Ohio

Bruno W. Volk, M.D.
Brooklyn, New York

ARNE N. WICK, PH.D., (*San Diego*): In your animal studies, Dr. Volk, did you find any changes in pancreatic alpha cells? It has been reported by some workers in Europe that there were changes in the alpha cells of the pancreas and in the liver and kidneys of guinea pigs which prolonged hypoglycemia.

BRUNO W. VOLK, M.D., (*New York City*): We have not been able to find any changes in the morphology of the alpha cells of the rabbit, indicative or even suggestive of damage.

GEORGES UNGAR, M.D., Sc.D., (*New York City*): Our experience was limited to phenformin dosages which would not produce hypoglycemic deaths in the experimental animals. No changes in the structure or function of the pancreas or any other organ of the animal were observed. We have also compared the toxicities of DBI and Synthalin A (decamethylene diguanidine) in guinea pigs and rabbits at doses producing equivalent hypoglycemic response (about ten times less Synthalin than DBI by weight). Twenty-four hours after administration of these agents, the animals were killed and both gross and microscopic studies of the kidneys and livers were made. There was invariably damage in the Synthalin-treated animals and no lesions at all in the DBI-treated animals. Our experience is limited to these observations.

DR. VOLK: We must consider that these drugs, although remotely related chemically, are really quite different. Also, there is a species difference that has to be considered. For instance, Creutzfeldt's description of kidney damage in animals, with DBI, was somewhat different from what we found. He observed tubular necrosis. One should not attempt to draw definite conclusions from a drug used in one species, and apply these conclusions to another, though related drug used in another species. Neither can the effect of one and the same drug necessarily be applied to different animal species.

MODERATOR WICK: Then, Drs. Volk and Ungar, you both feel there is a marked difference between Synthalin and DBI?

DR. VOLK: Yes, in the animals we have studied.

DR. UNGAR: Yes. In terms of animal toxicity there is a very considerable difference between them.

MODERATOR WICK: Is there any effect of DBI on the blood-forming tissues?

DR. UNGAR: We have observed no changes in bone marrow and spleen sections of the DBI-treated animal. There were no changes in the prothrombin levels in the control or the experimental animals and no changes in the over-all blood pictures; therefore we can say that in animals DBI has no untoward hematologic effects.

MAX MILLER, M.D., (*Cleveland*): Questions like this must, of course, be finalized in man. Many of the drugs commonly in clinical use today have no demonstrable toxic effect in animals, but in humans the so-called sensitivity reactions may prevail, so that the final answer must come from our observations in the patient.

Presented at the Symposium on "A New Oral Hypoglycemic Agent, Phenformin (DBI)" in Houston on Feb. 5, 1959.

I am thinking specifically of drugs, such as carbutamide, chlorpropamide, and even tolbutamide, which will produce sensitivity reactions in man, although no such reactions have been described in the animal.

SEYMOUR SHAPIRO, PH.D., (*New York City*): It has been reported by R. H. Williams et al. that liver glycogen is diminished under the influence of DBI. Dr. Volk's experiments on the other hand showed histologically that liver glycogen is unchanged. I wonder if the reported depletion of glycogen in fasted animals is an artifact, and actually does not reflect on glycogen storage in the human treated with DBI.

DR. VOLK: The histochemical determination of glycogen is a crude method. You may actually have more or less than you can demonstrate histochemically. Even in sections from different parts of the same liver, the histochemically demonstrable glycogen may vary considerably.

DR. UNGAR: We have done many glycogen determinations in controls and DBI-treated animals, and we found considerable depletion in fasting animals depending on the degree of starvation achieved. The extreme figures published by R. H. Williams were in fasting animals and after all, this is a consequence of starvation, not of DBI; in the animal with free access to food, glycogen depletion was not noted or was insignificant.

DR. VOLK: Our animals couldn't be kept fasting very well for up to three months. They ate daily. Even 20 per cent glucagon depletion cannot necessarily be demonstrated histochemically.

MARTIN G. GOLDNER, M.D., (*New York City*): Is it possible, Dr. Ungar, to withhold insulin from a diabetic monkey treated with DBI? If it is not possible with DBI, it would be very important data to present.

DR. UNGAR: The DBI-treated diabetic monkeys in our laboratories were kept controlled for two or three months until they died of respiratory infection. Our facilities for keeping monkeys were far from ideal and as is usually the case, their environment not their therapy eventually "caught up" with them. However, the diabetes in these animals was perfectly controlled to the end. Even when they contracted their infections, although we had to raise the DBI level, we always maintained them with less than 15 mg. of DBI per day.

DR. GOLDNER: I wouldn't have raised the question had we not had diabetic monkeys for some time (in 1943) which we kept alive for months on insulin.

MODERATOR WICK: We might go on to the next subject which is the mechanism of the action. The problem is, of course, to attempt to correlate the in vitro findings with those observed in vivo.

FRED A. KRUGER, M.D., (*Columbus*): Dr. Ungar mentioned that Nembutalized cats did not respond to DBI. It has been shown that Nembutal is an "uncoupling agent." I wonder how this affects our postulated mechanism.

DR. UNGAR: We observed that most general anesthetics, not only barbiturates, will decrease the hypoglycemic response of animals to DBI. We also tried other "uncoupling agents" both in vitro and in vivo, and the results were in agreement with Dr. Kruger's findings. We actually confirmed his observation that DBI in vitro inhibits oxidative phosphorylation. We can then speculate that Nembutal successfully competed with DBI in our studies in the cat. We also found that depending on the concentration of DBI used in our in vitro studies we could inhibit the DPN steps. These in turn were reversed by a number of chemicals particularly vitamin K (or K_1). In the in vivo studies vitamin K_1 was administered to the animals in considerable amounts and it did not influence the hypoglycemic effect of the DBI. It also decreased vomiting induced by DBI in these animals. Thus, although vitamin K could reverse the inhibition of the coupling of oxidation to phosphorylation by DBI in vitro, it had no effect on hypoglycemic response to DBI in vivo. The suppressive influence of vitamin K on vomiting induced in animals by DBI cannot be explained.

WILLIAM C. STADIE, M.D., (*Philadelphia*): I am quite confused and puzzled by discussions with respect to oxidative phosphorylation. For a long time it has been known that there appears to be a lack of formation of ATP in the absence of insulin in the completely diabetic patient. When insulin is added in vitro to liver or to muscle, it is quite possible to demonstrate increased formation of ATP from inorganic phosphate in the incubating media. In our laboratories, it was shown that a preparation of mitochondria prepared from the depancreatized cat by standard methods showed a distinct diminution of oxygen uptake as compared to similarly prepared mitochondria from livers of the normal cats. In addition to this there was a diminution of the ATP formation, so that the P/O ratio was markedly diminished, down to one. That meant essentially no phosphorylation.

Now, I am puzzled as to why DBI, a drug presumably intended for the treatment of a diabetic, would have precisely the opposite effect.

Thus, have studies been done on kidney mitochondria or liver mitochondria either in the normal or diabetic

cat to observe the effect of DBI, with respect to what seems to be an important aspect of the action of insulin?

DR. UNGAR: We have done P/O ratios in both liver and kidney mitochondria, and we did find a lowering of the P/O ratio in vitro with large concentrations of DBI. Naturally, this finding in vitro is puzzling since DBI, according to this concept would produce exactly the effect that the diabetes itself produced. I am also convinced it has no bearing whatever on the action of the drug. I somehow feel if you put a large enough amount of any drug into any enzyme system, you will inhibit it, and since these are the most worked-on enzymes of those that we know, these have been shown the most frequently inhibited by drugs of all categories.

JOSEPH LARNER, M.D., (*Cleveland*): I have not yet seen the data on the P/O ratios, and yet these ratios are the key to the mechanism involving oxidative phosphorylation. What was the substrate? What were the depletions? And the actual oxygen consumption? And also, when you say that DPN "bypassed," what happened to the P/O ratios?

DR. KRUGER: I would like to discuss not our own work, but the published work of Hollunger. We have not measured P/O ratios. Dr. Ungar has and probably will add to this, but according to Hollunger, P/O ratios do not change with guanidines, just the total oxygen uptake and total phosphorylation. The rate determining step has always remained the same. In other words, you cannot reduce phosphorylation without also lowering electron transport unless you have a "bypass," and he actually found P/O ratios not changed; the total oxygen uptake and the total phosphorylation were diminished in proportion to each other. Now, the uncoupling agent made the P/O ratio zero both in the presence and absence of the guanidine compound. This is different from what Dr. Ungar has found.

DR. SHAPIRO: Dr. Kruger is referring in his comments to Hollunger's work with guanidines. As Dr. Ungar has already pointed out, even with closely related biguanides, there are considerable differences in the types of activity. I don't think therefore that it is valid to refer to mechanisms of action suggested for guanidines or guanidine itself since these may not at all relate to the responses effected with a biguanide, DBI. I think we lose sight of our objective, which is to define the action of DBI when we refer to mechanisms of action which are so nonspecific that a variety of compounds can produce the same kind of in vitro effects, but do not produce the same in vivo responses. Let it be understood that guanidines, diguanidines and biguanides may sound alike, but they are chemically and apparently, physiologically, vastly different.

DR. UNGAR: The P/O ratio does have meaning when you deal with uncoupling agents. We have reduced P/O ratios with DBI to less than one, but I do not know whether this means very much. DBI at some concentrations in vitro, does inhibit phosphorylation as shown by changes in the uptake of organic phosphate and its release under various conditions. With an uncoupling agent, reversal is not complete. You have much less inhibition of oxidation in the presence of the uncoupling agent which suggests that the inhibition of oxidation is caused by the inhibition of phosphorylation, the block being exactly at the point Dr. Kruger located it. I would, however, like to emphasize that this is purely an in vitro finding, and although we have tried to show this in vivo, even when DBI has lowered the blood sugar to nearly zero, we were not able to do so.

DR. MILLER: Dr. Ungar has emphasized that the concentrations of DBI in the in vitro experiments may have nothing to do with the concentrations required to produce a clinical effect. Are there methods for determining DBI in body fluids, and if so, what concentrations are present in body fluids that would coincide with a hypoglycemic effect?

DR. SHAPIRO: In our analytical laboratories, methods have been developed by which we can isolate DBI and separate it from the other groups interfering with analytical procedures. By these methods it is possible to determine concentrations of DBI of about 5 gamma/cc., which unfortunately may not be adequate for work in man at this point, but should be very instructive in doing work with animals. It is my understanding that these methods will soon be described in the literature.

DR. MILLER: Are you implying that the concentrations in man that produce hypoglycemia are less than 5 gamma?

DR. SHAPIRO: If man responds to 100 mg. by a very loose calculation, he would have about 1 mg. per liter distributed in the blood and tissue and that would be roughly one gamma/cc. Thus, I would say that our analytical technics should be that sensitive. The present method is not.

MODERATOR WICK: DBI appears to be concentrated in certain organs, such as the liver. We know that the effects of DBI last over six hours at least, and during this time, the drug certainly must be present in the body in proportion to where its carbon^{14} label is. Labeled DBI was given, therefore, wherever the carbon^{14} is; it is present either as DBI or its breakdown in relative proportions.

I would, however, like to point out that we do not know if we are measuring the effect of the drug on

the body or the body on the drug at the present time.

DR. SHAPIRO: In the clearance work done by us, we did find large amounts of DBI or its metabolite in the urine: It was established that the material excreted in the urine gives all the color tests for DBI; thus Dr. Wick's apprehension that he is measuring something other than DBI, at least in the urine, does not seem too well founded.

DONALD W. CLARKE, PH.D., (*Toronto*): In regard to the criticism of dosage which was raised earlier by Dr. Ungar, in working with in vitro technics, it is recognized that you cannot directly transfer the effect from a liver slice to a whole animal. One must make allowances that you are working with an artificial system; the tissues isolated from the animal might not be expected to respond to a drug at the same dose as would be required in the whole animal. You might expect reasonably that the isolated tissue would require a rather higher dose of the drug. Take, for example, the comparison of insulin doses given to the intact animal and introduced into the isolated diaphragm. The isolated diaphragm, unless great care is used, may require a hundred milliunits while the whole animal will respond to one milliunit of insulin per milliliter of blood. It does seem to suggest that the criticism of the high dosages might be subject to a certain amount of revision for this is quite standard in in vitro tests. It would appear that you do have to use higher dosages.

MODERATOR WICK: To return to the consideration of intermediary metabolic effects of DBI, is it agreed that lactate levels increase in animals and in vitro tissue preparations upon introduction of DBI? If so, how then does that lactic acid get there? And how is it disposed of?

DR. UNGAR: I agree that there is an increase in lactate in the animal in vivo, but not necessarily more than with intravenous insulin.

DR. KRUGER: I would not agree with that. Lactate levels in patients on insulin do not rise above normal. They do with DBI therapy, two or three times!

COMMENT FROM FLOOR: With or without DBI, we get increased lactate levels. These vary from patient to patient. I would like to point out, however, that in our studies we get increases in both pyruvate and lactate with the DBI, which greatly exceed those of diabetic patients on insulin.

DR. KRUGER: This brings up the question of differential tissue location; whether you are making lactate in one organ and disposing of it in another. This ties in with the localization of the action of DBI, about which we do not yet know the final answer. There may be production in a certain organ, and utilization elsewhere. We did lactate determinations in blood from hepatic veins, femoral arteries, and peripheral veins on people who were on DBI, and found them all elevated, but not consistently so. We couldn't show in which way lactate was going.

DR. WICK: Are we exchanging the lactate carbon for glucose carbon?

DR. STADIE: Could you tell us the source of this lactic acid? Has that been determined in the intact human?

DR. KRUGER: Organ-wise, no, not to my knowledge.

DR. STADIE: In vitro, or in animals, what is the source?

DR. KRUGER: In diaphragm, the lactate may come from the glucose or the glycogen. You get marked uptake of glucose and marked increase of lactate in the incubating media.

DR. UNGAR: We have some contradictory data on the lactic acid story. In vivo, as seen in slides shown earlier, we find that contrary to the classic concept increased amounts of lactic acid are produced in the liver and are disposed of in the muscle. In vitro, however, we find the opposite, that is, that DBI will increase the lactic acid in the muscle preparation and inhibit its disposal in this tissue. I don't know how to reconcile these contradictory data.

MODERATOR WICK: I think current ideas about lactic acid metabolism will have to be revised. We now know that lactic acid is oxidized readily by muscle tissues, oxidized at a rate perhaps two or three times that of the rate of glucose utilization.

DR. LARNER: In regard to Dr. Stadie's question, I don't believe it has as yet really been determined how much lactate comes from muscle glycogen breakdown and how much from increased glucose uptake. It would be ideal to be able to prelabel muscle glycogen, then introduce DBI and "cold" glucose and study the output of lactate. From the radioactivity of the lactate one could estimate how much comes from glycogen and how much from glucose.

DR. KRUGER: It has been observed that glycogen in the diaphragm after DBI is way down. We also know that oxygen uptake is way down, thus the glucose taken up is not being burned. We know the lactate level goes way up. So, some lactate must come from the glycogen, and all the glucose taken up supplies the rest, since none of it can be going to glycogen.

MODERATOR WICK: Is it not just possible that the glucose taken up does go to glycogen and then to lactate as the process of glycogenolysis in the presence of these high concentrations of DBI takes place? We

should not overlook this possibility.

LEONARD L. MADISON, M.D., (*Dallas*): I would like to mention one essential fact regarding these comments on mechanism. While DBI works in diabetic and nondiabetic animals, it apparently has no effect on nondiabetic humans. Should we not consider the possibility that it may not at all be justifiable to attempt to relate in vitro and in vivo observations with animals to the influence of DBI on humans. No one has yet shown quantitatively that you can correlate the disappearance of glucose with the appearance of lactate. Also, if lactate were being traded for glucose without the bodily utilization of the lactate, then the lactate should be excreted nearly quantitatively in the urine. Apparently little lactate is excreted, thus it is being utilized and in this case respiration must be going on.

In preliminary experiments (and these are few in number) we have been able to show a one-to-threefold increase in the utilization of glucose labeled with carbon^{14}, over the hexose monophosphate shunt, thus we have evidence suggestive of increased utilization of glucose, and this, of course, by an oxidative pathway which would give a hypoglycemic effect.

DR. WICK: It is apparent that one can easily demonstrate in a clear fashion an effect of DBI on specific areas of metabolism when using in vitro experiments. However, it is difficult to correlate these in vitro observations with the known hypoglycemic action of DBI. Perhaps the relatively high concentrations of DBI employed in the in vitro experiments produce metabolic effects of a "forcing" nature which cannot be detected or do not occur under in vivo physiological conditions. On the other hand, probably too little attention has been focused on the alternate pathways for the utilization of glucose. In this connection, the preliminary findings of Madison suggesting that glucose utilization by the hexose monophosphate shunt is increased by DBI encourage further investigation. It is apparent that caution must be exercised in assuming that a biochemical reaction occurring in vitro will occur also in vivo.

Chemical Basis of Heredity

Perhaps the most revolutionary achievement of the recent period of our history has involved the chemical basis of heredity. It had been known since Miescher's work in the 1870's that chromosomes (of fish sperm) are largely composed of protein and nucleic acid. Until rather recently, nucleic acid seemed ruled out as the site of specificity by its supposedly monotonous tetranucleotide structure. Most geneticists up to the middle 1940's assumed that there was little choice but to suppose that specificity resided in a pattern of successive amino acids. Then came the discovery that the pneumococcus transforming principle, acting like a free gene, was probably pure DNA, and the recognition by biochemists that there was after all an adequate basis for specificity in the sequence of nucleotides in chains of indefinitely great length. The demonstration of constancy of DNA per chromosome set added to the confidence in its genetic significance.

There was a transition period when the conjecture of a reciprocal relation between protein and DNA, each imposing specificity in synthesis of the other, seemed most attractive, but soon Watson and Crick gave us a model of DNA which indicated a reciprocal relation between two intertwined complementary but oppositely directed strands of this material itself. As this model was based on precise analysis of the X-ray diffraction pattern, it marks a tremendous step beyond mere conjecture toward understanding the actual chemistry of heredity.

This, of course, is only a beginning. The problem of the untwisting of the complementary strands is one that is difficult to contemplate without vertigo, at least if it must proceed through the whole length of the chromonema, with its hundreds of thousands of gyres. The difficulty would, of course, be mitigated if the DNA were broken up without disruption of order into units with molecular weights of perhaps 10^6 (some 150 gyres) all capable of untwisting simultaneously.

The problem of how DNA imposes patterns on other macromolecules (RNA, proteins, polysaccharides) has not yet advanced beyond the stage of conjecture. Especially disconcerting is the fact that some viruses have only RNA and protein and no DNA at all.

From "Genetics and the Hierarchy of Biological Sciences," by Sewall Wright, in *Science*, Vol. 130, No. 3381, pp. 959-65, Oct. 16, 1959.

Clinical and Metabolic Effects of Phenethylbiguanide

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Phenethylformamidinyliminourea hydrochloride (DBI) is a nonsulfonylurea compound capable of lowering the blood sugar when administered by mouth. Its hypoglycemic action has been demonstrated in human subjects in a variety of animal preparations including the eviscerate guinea pig,[5] alloxan-diabetic rabbits, rats and monkeys.[3] Death in animals following DBI administration has resulted from hypoglycemia[1] and cardiac arrhythmias[32] but, to date, no fatalities in man have been attributed to the drug and no cases of renal, hepatic or hematopoetic toxicity have been reported in patients receiving the drug.

MATERIALS AND METHODS

Forty-two patients with diabetes mellitus were treated with DBI for a minimum of one month, or until side effects precluded further administration of the drug. All patients were hospitalized, placed on a constant diabetic diet and evaluated for renal, hepatic and hematopoetic abnormalities. If they were on insulin, their current requirement was stabilized. Blood glucose was determined by the Somogyi-Nelson technic[28] and nonesterified acids by the method of Dole and his associates.[14]

The patients in this series ranged from sixteen to eighty-five-years in age. Four were juvenile-type diabetics, and nine showed marked changes in the level of their blood sugar from day to day and thus were considered to fall into the "labile" category. Duration of diabetes in these patients ranged from a few days to twenty-two years. Twenty-five of the group required 10 to 85 U. of insulin per day for control of hyperglycemia, while seven had unsuccessfully attempted dietary control. Response to DBI was graded as follows:

Good—fasting blood sugar 60-150 mg. per 100 ml., no glycosuria over two plus.

Fair—fasting blood sugar 150-200 mg. per 100 ml., no glycosuria over three plus.

Poor—fasting blood sugar over 200 mg. per 100 ml., frequent glycosuria over three plus.

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From the Department of Medicine, St. Luke's Hospital, New York 25, New York.

RESULTS

In twenty patients hyperglycemia was controlled by DBI alone, while thirteen patients (including six of the labile type) required less insulin than they had prior to DBI administration. In these patients diabetic control seemed to be stabilized by addition of the drug. Of the individuals who received DBI alone thirteen obtained good control, three fair control and four poor control according to the criteria already described. Ten of the thirteen patients receiving DBI and insulin maintained good and three fair control of their hyperglycemia.

Eight patients developed untoward side effects before significant control of hyperglycemia was attained and therefore no further DBI was administered. Sixteen patients who were successfully treated also developed side effects, as follows: nausea in ten, vomiting in six, diarrhea in two, metallic taste in one, and dizziness in one. Two patients showed intermittent acetonuria while taking DBI and maintaining adequate caloric intake. In ten patients who developed side effects, therapy was discontinued, while in six it was possible to minimize side effects and still control hyperglycemia by reducing the dose (table 1). Twenty-four of the series are currently receiving the drug. Nineteen are maintaining good control and five fair control. Two patients on insulin plus DBI have reported episodes suggesting hypoglycemic attacks. Unfortunately, chemical confirmation was not obtainable in either patient.

One patient in the series had undergone a total pancreatectomy for chronic pancreatitis. As can be seen in table 2, DBI appears to have reduced this patient's hyperglycemia despite lack of endogenous insulin and reduction in the dose of exogenous insulin. In this subject, twenty-four-hour urine glucose excretion decreased during DBI treatment, but on the last day of treatment marked anorexia developed, and since constant caloric intake could not be maintained the study had to be terminated.

In an attempt to elucidate the mode of action of the biguanides, the acute effect of DBI on plasma nonesterified fatty acids (NEFA) was studied in human subjects. NEFA levels are known to be lowered by ingestion of carbohydrate,[15,16] tolbutamide,[17] glucagon[34] and amino

TABLE 1
Results of therapy with DBI

	Number of patients		
Adequate period for evaluation	42		
DBI alone	20		
Control—good		13	
fair		3	
poor		4	
DBI and insulin	13		
Control—good		10	
fair		3	
Failures	9		
Side effects prior to therapeutic effect		8	
Total pancreatectomy		1	
Side effects (including failures)	20		
DBI stopped		14	
Loss of side effects on smaller dose		6	
Gastrointestinal			4
Acetonuria			2

TABLE 2
J.M—St. Luke's Hospital #358-573. Total pancreatectomy, April 1957

Date	Weight (lb.)	NPH Insulin (units)	Reg. Insulin (units)	DBI mg.	FBS mg. per 100 ml.	Total twenty-four hr. gm. glucosuria
7/8	133	40	10-20	none	287	67.2
7/9	133	40	15	none	150	36.9
7/10	135	40	20	none	188	52.2
7/11	136	20	15	50	206	5.1
7/12	—	20	10	75	200	73.2
7/13	136	20	5	75	287	64.0
7/14	—	15	15	125	—	11.7
7/15	—	15	20	175	312	25.0
7/16	136	10	10	175	250	9.7
7/17	—	10	5	225	262	5.9
7/18	136	40	15	Stop	300	—

acid administration.[18] It would appear that NEFA are mobilized from adipose tissue[19] to supply energy needs when glucose utilization is decreased.[14,20,21]

In the poorly controlled diabetic patient, plasma NEFA tend to be abnormally elevated and, like the blood sugar, fall when insulin is administered.[14] Intravenous administration of tolbutamide, in responsive patients with diabetes mellitus, also induces a drop in plasma NEFA concentration.[17]

Accordingly, plasma NEFA levels were followed in diabetic patients (previously determined to be responsive to DBI) after administration of the biguanide. It was reasoned that a significant decrease in plasma NEFA after DBI might provide evidence for an increase in glucose utilization induced by the drug. These subjects all had mild diabetes mellitus and had received no DBI for one week, no depot insulin for forty-eight hours, and no crystalline insulin for at least twelve hours prior to the test. All were studied in the postabsorptive state and were given oral glucose (1.0 gm./kg. body weight) on a control day, and the same amount of glucose plus 50 to 100 mg. of DBI on the test day. Venous blood specimens were taken prior to administration of glucose and/or DBI one, two, three, four and five hours subsequently.

At the dose level of 50 mg. of DBI, the four patients tested showed no difference in NEFA or blood glucose response to the glucose load. When the dose of DBI was increased to 100 mg., an increase was noted in the rate of fall of NEFA concentration in plasma, although no consistent changes in the blood sugar occurred.

DISCUSSION

Numerous studies have indicated that the biguanides promote anaerobic glycolysis.[22–24] DBI may have such an effect by blocking one or more enzyme systems upon which the citric acid cycle depends.[24,25] The lack of any effect of DBI on plasma and urine steroid levels, or ACTH responsiveness[26,27] would seem to eliminate interference with gluconeogenesis as an action of the biguanides. Thus far studies with glucagon and epinephrine have been contradictory but neither hormone seems to be involved in the mode of action of DBI.[11,27]

The increased uptake of glucose by muscle that has been reported after DBI administration[4,5] suggests that phenethylbiguanide may increase peripheral glucose utilization under certain conditions.[28] Studies by Lundbaek[10] and Nielson et al.[5] have shown DBI to be effective in alloxan-diabetic and pancreatectomized animals. The results obtained in our patient with a total pancreatectomy must be considered inconclusive.

In the series presently reported, DBI helped in the control of hyperglycemia in approximately 60 per cent of the patients with diabetes mellitus who were given a therapeutic trial. If the patient does not develop side effects, there seem to be two main groups in which DBI may be a useful agent: patients requiring small amounts of insulin for regulation and patients who have wide swings in blood sugar from day to day. In this latter group the biguanides seem to smoothe the course.[7–9,11] In the present series, four patients were studied who fell into this category. One was controlled by DBI alone, two had their diabetic state stabilized by the drug, and the fourth was unable to tolerate it in a dose sufficient to alter his hyperglycemia.

The over-all incidence of side effects of DBI is between 35 and 60 per cent.[6–9] These symptoms are so unpleasant or occur at such low dosage in about 15 to 35 per cent as to preclude further attempts at DBI management. The use of the arylsulfonylureas and biguanides jointly, as suggested by Beaser[29] may circumvent some side effects, but until this handicap can be overcome, the extent of biguanide control of hyperglycemia cannot be fully evaluated.

SUMMARY

The effect of phenethylbiguanide on hyperglycemia was studied in forty-two patients with diabetes mellitus. Twenty were placed on the drug alone and of this group thirteen achieved good control of hyperglycemia, according to previously established criteria. Thirteen patients were given it as a supplement to insulin and, of these, ten obtained good control of their blood sugar levels. Fourteen patients developed gastrointestinal side effects which prevented further biguanide therapy, while six others with similar complaints were able to tolerate lower doses and maintain adequate control. The drug seems to be most effective in mild diabetes, or as an adjunct to insulin treatment in the labile patient.

SUMMARIO IN INTERLINGUA

Effectos Clinic e Metabolic de Phenethylbiguanida

Le effecto de phenethylbiguanida super hyperglycemia esseva studiate in quaranta-duo patientes con diabete mellite. Vinti recipeva solmente phenethylbiguanida. Dece-tres de illes obteneva un bon grado de stabilisation de lor hyperglycemia secundo previemente establite criterios. Dece-tres altere patientes recipeva phenethylbiguanida como supplemento de insulina. In dece de istes, bon grados de stabilisation del nivellos de sucro sanguinee esseva effectuate. Dece-quatro patientes disveloppava effectos secundari gastrointestinal que preveniva le continuation del therapia a phenethylbiguanida. Sex alteres, con simile gravamines, esseva capace a tolerar plus basse doses e mantener assi un stabilisation adequate. Phenethylbiguanida es le plus efficace in le patiente con leve grados de diabete mellite.

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Influence of Phenethylbiguanide on Lactic, Pyruvic and Citric Acids in Diabetic Patients

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The biochemical effects of the oral hypoglycemic agent DBI (phenformin, phenethylbiguanide) in experimental animals have included alterations in the metabolism of lactic acid as well as of glucose. DBI not only increases the glucose uptake of the rat diaphragm in vitro but also decreases the oxygen uptake and increases the lactic acid production of that tissue.[1] Lactic acid production by the guinea pig liver slice is also increased by the addition of DBI.[1] In the same animal an elevation of the blood lactic acid concentration has been observed after administration of the drug.[1] Accordingly, it appeared of interest to determine whether or not lactic acid metabolism was also altered in diabetic patients who were given DBI.

The present studies on diabetic patients have included an investigation of the effects of DBI on (1) the concentration of lactic acid in the fasting blood, (2) the twenty-four-hour urinary excretion of lactic acid, (3) the changes in the blood lactic acid concentration after the administration of glucose or fructose, potential precursors of lactate, and (4) the changes in the blood lactic acid concentration after sodium lactate administration. Since lactate is in equilibrium with pyruvate, changes in lactic acid cannot be properly interpreted without a knowledge of the associated pyruvic acid changes.[2] Therefore, both pyruvic and lactic acid concentrations were measured in the blood and urine specimens. In addition, changes in the blood concentrations and urinary excretions of glucose and citric acid were studied.

Presented at the Symposium on "A New Oral Hypoglycemic Agent, Phenformin (DBI)" in Houston on Feb. 5, 1959.

Preliminary results of these studies were presented to the Cleveland Section of the Society for Experimental Biology and Medicine on April 21, 1958.

METHODS

The subjects for these studies were eight diabetic patients whose clinical characteristics are shown in table 1. They were hospitalized on the Metabolic Research Division of the University Hospitals of Cleveland where they received constant diets. Each subject was studied during an initial control period of three to seventeen days and a six- to forty-eight-day period of DBI administration. In some cases observations were continued during a final control period of seven to ten days after DBI administration was discontinued.

During the control periods six of the patients received no insulin; patient J.C. was maintained on a reduced amount of regular insulin which was given in two doses daily and was continued during the period of DBI administration; during the preliminary control period patient O.G. received Protamine Zinc Insulin in an amount adjusted to give a similar degree of blood sugar lowering to that which was subsequently produced by DBI. DBI* was administered orally in four doses daily with meals and the bedtime feeding; the total daily dose varied between 100 and 175 mg. In two patients DBI was also given rectally in suppositories; the maximum daily dose by this route was 300 mg. in one case and 400 mg. in the other. Fasting venous blood samples were obtained daily before the patient arose. Daily twenty-four-hour urine samples were collected; no preservative was used, but the urine was refrigerated during collection. In order to determine the stability of the urinary lactic and pyruvic acids under these conditions, the concentrations of these acids were determined in urine immediately after it was voided and again twenty-four hours later. No significant change in lactic or pyruvic acid content occurred.

Glucose, fructose, and lactate tolerance tests were per-

* Supplied by Dr. Harvey S. Sadow, U. S. Vitamin & Pharmaceutical Corporation.

TABLE 1

Clinical information on diabetic patients

Subject	Age	Sex	Height	Weight	Diabetes		Hypoglycemic agents used previously
					Duration	Type	
	Yr.		Cm.	Kg.	Yr.		
J.C.	46	M	180	63	21	Ketosis-prone	Insulin-40 U*
C.T.	70	M	170	55	4	Stable	None
G.P.	68	F	165	68	8	"	Insulin-12 U*
H.F.	51	M	166	56	1/4	"	None
R.B.	51	F	175	68	1/6	"	None
A.J.	64	F	156	85	11	"	Insulin-220 U*
O.G.	58	M	175	85	2	"	Tolbutamide† Chlorpropamide†
H.M.	38	F	165	61	1	Ketosis with infection Insulin allergy	Insulin-30 U* Tolbutamide†

*Daily insulin dose in units.
†Therapeutically ineffective.

formed during the control period and were repeated while the patients were receiving DBI, the last dose of DBI being given approximately six hours before the beginning of the test. The tolerance tests were performed with the patient resting in bed after an overnight fast. An infusion pump was used to administer the test substance intravenously at a constant rate over the period of one hour. One gram per kilogram of body weight of glucose or fructose was given as a 10 per cent solution in water, while 1.36 mM. per kilogram of sodium r-lactate as a one-sixth molar solution in water was used.* A control venous blood specimen was drawn and samples were then obtained at thirty-minute intervals after the beginning of the infusion for a total of three hours; an indwelling needle was used and samples were obtained without a tourniquet in most cases.

The following methods of analysis were applied to the blood and urine samples: the method of Somogyi[5] for glucose; the method of Friedemann and Haugen[4] for pyruvic acid; the method of Barker and Summerson[6] for lactic acid; a combination of the methods of Natelson, Pincus, and Lugovoy[6] and of Taussky and Shorr[7] for citric acid; and the method of Higashi and Peters[8] for fructose after the removal of glucose by glucose oxidase. Analysis of urine to which known amounts of lactic acid had been added yielded the following average recoveries at different levels of lactic acid concentration: 5 mg. per 100 ml.—83 per cent recovery; 10 mg. per 100 ml.—94 per cent; 50 mg. per 100 ml.—95 per cent; and 100 mg. per 100 ml.—100 per cent.

* Parenteral solutions used in these studies were supplied by Dr. E. A. Hawk of Mead Johnson.

RESULTS

Fasting blood concentrations of metabolites (table 2 and figure 1): DBI lowered the fasting blood glucose concentration in all subjects; an effect was observed with 100 mg. daily, which was the smallest dose utilized, and the hypoglycemic effect increased progressively as the dose was raised to 175 mg. daily, the maximum dose employed. DBI administration was accompanied by an increased concentration of lactic acid in

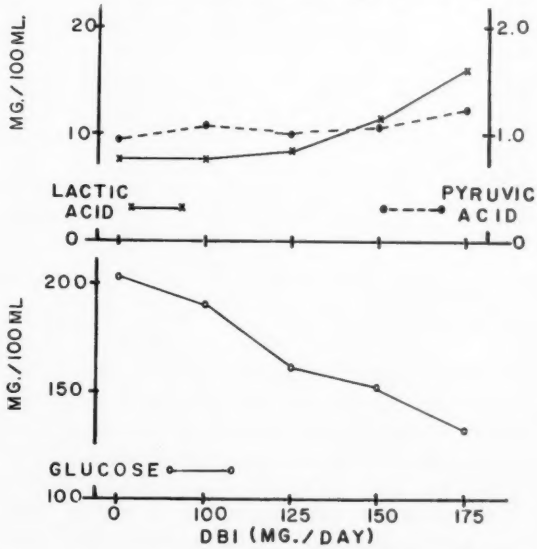

FIG. 1. Influence of DBI on the average fasting blood concentrations of lactic acid, pyruvic acid, and glucose in six diabetic patients (G.P., H.F., R.B., A.J., O.G., and H.M.). The pyruvic acid concentrations are plotted on a scale ten times as great as the lactic acid scale.

TABLE 2

Effect of DBI on average fasting blood concentrations and twenty-four-hour urinary excretions of metabolites in diabetic patients

Patient	Number of days	Insulin (units)	DBI (mg.)	Fasting blood or plasma				24-hour urinary excretion			
				Glucose	Pyruvic acid (mg. per 100 ml.)	Lactic acid	Citric acid	Glucose (gm.)	Pyruvic acid (mg.)	Lactic acid (mg.)	Citric acid (mg.)
J.C.	6	50	0	248				3.4			
	7	25	0	425				41.5			
	8	25	150	212		31.4		5.2			
	9	15	150	282		31.3		7.1			
	7	25	150*	431				59.2			
	5	25	300*	569		11.8		90.0			
	2	25	0	586		4.0		85.4			
C.T.	6	0	0	152		5.9	2.3	3.8			
	13	0	150	112		11.8	3.1	0.9			
	9	0	0	154		6.3	2.6	5.1			
G.P.	4	0	0	168	0.95	8.0		9.1		43	
	4	0	100	156	0.91	5.5		3.8		59	
	2	0	150	142	1.03	14.8		2.7		594	
H.F.	7	0	0	151	0.79	9.5	1.9	7.4	21	84	594
	5	0	100	133	1.18	7.2		1.7	23	88	978
	18	0	125	104	1.10	13.4	1.8	0.6	24	115	925
	4	0	175	91	1.10	16.0	2.5	0.5	21	91	1,208
	7	0	400*	114	1.32	14.1		1.0	23	92	1,223
	6	0	0	119	1.04	15.2	1.9	0.8	19	73	966
	4†	0	0	169	0.84	11.9		3.7	25	111	1,312
R.B.	8	0	0	254	0.82	9.4	3.1	11.5	27	90	509
	4	0	100	213	0.82	8.8		5.6	23	87	763
	4	0	125	156	0.78	8.2		1.3	23	85	488
	4	0	150	143	1.08	10.9		0.8	24	99	689
	4	0	175	113	1.11	22.6	3.8	0.7	42	207	880
A.J.	5	0	0	237	1.05	6.5	3.0	16.5	42	62	800
	3	0	100	220	1.51	10.0		8.6	43	98	984
	5	0	125	165	1.33	8.5		2.3	35	67	764
	4	0	150	151	1.18	11.2		1.2	42	118	624
	6	0	175	141	1.63	18.5	2.9	1.1	49	227	499
O.G.	6	40	0	108	1.02	5.7	1.8	6.9	23	48	995
	2	0	100	170	0.95	7.8		6.0	24	47	1,362
	3	0	125	154	1.07	7.2		1.5	21	33	1,274
	4	0	175	113	1.16	9.4	2.4	1.1	34	94	1,680
H.M.	3	0	0	301	0.99	6.5		80.6	33		
	2	0	100	250	1.10	6.1		65.9	24	82	
	2	0	125	241	0.80	4.6		45.2	30	104	
	2	0	150	226	0.94	8.7		28.1	47	216	
	4	0	175	202	1.16	12.2		10.0	61	374	

*Suppositories.
†One month later.

the fasting blood in all patients. A daily dose of 100 mg. of the drug produced no unequivocal increase in the blood lactic acid level even though the fasting blood glucose concentration was diminished. At the dose level of 175 mg. daily the average fasting blood lactic acid concentration was more than double the control value. The delay in the decline of the elevated blood lactic acid level after discontinuation of DBI in subject H.F. is unexplained. Although the fasting blood pyruvic acid concentration increased during DBI administration, the increases were proportionately less than the lactic acid changes; the average maximum increase was less than 50 per cent of the initial value. As a result, the lactate: pyruvate ratio was increased. The administration of DBI by the rectal route had little or no effect on the concentration of glucose and other metabolites in the fasting blood. In subject O.G. lowering of fasting blood sugar by daily administration of Protamine Zinc Insulin was not accompanied by a rise in blood lactic acid concentration or an increase in the lactate:pyruvate ratio. DBI produced slight increases in the average fasting plasma citric acid concentration in four of five subjects.

Urinary excretion of metabolites (*table 2 and figure 2*): The twenty-four-hour urinary excretion of glucose, lactic acid, pyruvic acid, and citric acid reflected the changes in their concentrations in the blood. Glucose excretion decreased progressively in all patients as the dose of DBI was increased. The lactic acid excretion was increased twofold or more in five of six subjects and the pyruvic acid excretion increased moderately in three of five subjects. The increases in lactic acid excretion were equivalent to only a small fraction of the observed decreases in glucose output. The increases in lactic acid excretion were equivalent to only 0.5 to 1.1 per cent of the decreases in glucose output except in the case of subject G.P. whose urinary lactate increase was equivalent to 8.6 per cent of the urinary glucose decrease. There was an increased excretion of citric acid in three of four subjects.

Glucose tolerance tests (*table 3*): In two of the three subjects tested the maximum rise in blood glucose produced by glucose administration was less when the patients were receiving DBI. The blood lactic and pyruvic acid changes were small and the effect of DBI on these changes was variable.

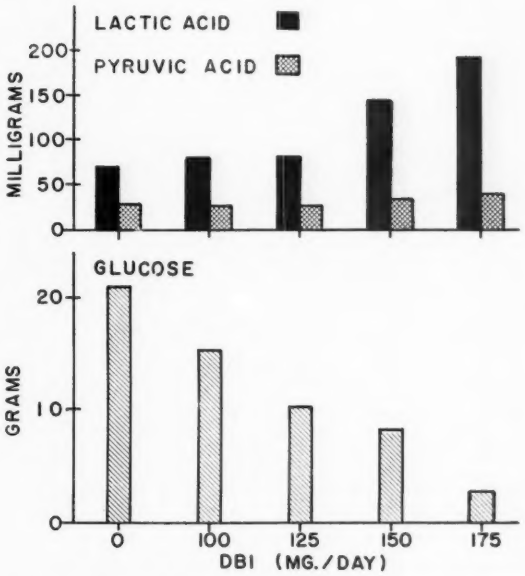

FIG. 2. Influence of DBI on the average daily urinary excretions of lactic acid, pyruvic acid, and glucose in six diabetic patients (G.P., H.F., R.B., A.J., O.G., and H.M.).

TABLE 3

Influence of DBI on the changes produced in the blood concentrations of metabolites by glucose administration* in diabetic patients† (mg. per 100 ml.)

Subject	DBI therapy	Fasting	Change from fasting: Minutes after start of glucose infusion 30	60	90	120	150	180
					Blood lactic acid			
C.T.	Before	6.4	1.4	2.2	1.4	1.1	1.2	0.8
	During	19.6	2.8	2.4	2.3	2.6	—0.4	—1.4
H.F.	Before	14.7	—1.6	—2.7	—5.6	—6.2	—6.2	—6.3
	During	22.0	—0.3	—2.2	—2.5	—1.7	—2.1	—1.0
R.B.	Before	11.9	0.9	3.0	4.4	4.6	2.1	—0.2
	During	26.8	—1.3	—6.7	—5.9	—6.0	—9.8	—12.3
Mean	Before	11.0	0.2	0.8	0.1	—0.2	—1.0	—1.9
	During	22.8	0.4	—2.2	—1.9	—1.7	—4.1	—4.9
					Blood pyruvic acid			
C.T.	Before	1.04	—0.18	—0.29	—0.08	0.11	0.08	0
	During	1.28	0.14	0.22	0.27	0.11	—0.02	—0.13
H.F.	Before	0.78	—0.16	—0.19	—0.16	—0.14	—0.13	—0.06
	During	0.70	—0.11	0	—0.06	—0.19	—0.16	—0.03
R.B.	Before	0.56	0.08	0.08	0.35	0.30	0.14	0.22
	During	0.56	0.08	—0.08	0	0.06	0.43	0.64
Mean	Before	0.79	—0.09	—0.13	0.04	0.09	0.03	0.05
	During	0.85	0.04	0.05	0.07	—0.01	0.08	0.16
					Blood glucose			
C.T.	Before	157	139	257	195	170	143	131
	During	114	139	229	168	135	77	56
H.F.	Before	140	172	248	199	149	106	73
	During	91	105	208	163	99	52	33
R.B.	Before	236	130	232	170	100	76	97
	During	115	146	243	203	182	148	141
Mean	Before	178	147	246	188	140	108	100
	During	107	130	227	178	139	92	77

*One gram of glucose per kilogram was administered intravenously in one hour.
†Unless indicated otherwise, values are positive.

Fructose tolerance tests (table 4 and figure 3): In two cases the maximum rise in the blood fructose level was somewhat less when subjects were receiving DBI, and in three cases the blood glucose increase was less during therapy with the drug. In all five patients the increase in the blood lactic acid concentration after fructose administration was greater when the patients were receiving DBI with an average maximum increase of

TABLE 4

Influence of DBI on the changes produced in the blood concentrations of metabolites by fructose administration* in diabetic patients† (mg. per 100 ml.)

Subject	DBI therapy	Fasting	Change from fasting Minutes after start of fructose infusion					
			30	60	90	120	150	180
					Blood lactic acid			
C.T.	Before	4.9	15.1	23.7	14.8	10.3	4.2	2.5
	During	16.1	19.7	36.8	24.3	13.0	10.8	6.6
H.F.	Before	9.9	19.4	20.8	12.1	4.3	2.8	0.1
	During	18.5	16.0	29.5	14.1	2.3	—3.3	—4.4
R.B.	Before	8.2	31.3	49.2	43.4	26.5	15.5	10.2
	During	23.1	33.3	59.9	41.1	33.8	21.6	11.9
A.J.	Before	4.9	22.1	39.0	24.7	12.6	5.8	3.1
	During	12.8	38.8	57.9	36.5	26.8	18.1	15.0
O.G.	Before	4.5	28.4	30.1	17.6	10.2	6.9	4.7
	During	12.1	32.6	46.6	25.4	15.0	8.8	7.1
Mean	Before	6.5	23.3	32.5	22.5	12.8	7.0	4.1
	During	16.5	28.1	46.1	28.2	18.1	11.2	7.2
					Blood pyruvic acid			
C.T.	Before	0.67	0.83	1.07	0.85	—0.05	0.08	0.35
	During	1.26	0.08	0.88	0.85	0.45	0.26	0.02
H.F.	Before	0.51	0.08	0.53	1.09	0.40	0.32	0.48
	During	0.64	0.03	0.22	0.30	—0.02	—0.08	0.06
R.B.	Before	0.88	0.51	1.21	1.05	0.97	0.54	0.51
	During	1.39	0.51	1.87	0.67	0.70	—0.03	0.21
A.J.	Before	0.86	0.88	0.96	2.54	1.84	1.04	0.77
	During	1.28	2.89	3.05	2.30	1.66	1.05	0.53
O.G.	Before	1.12	0.27	0.38	0.62	0.30	0.16	—0.05
	During	1.20	3.00	3.00	0.81	0.83	0.59	0.51
Mean	Before	0.81	0.51	0.83	1.23	0.69	0.43	0.41
	During	1.15	1.30	1.80	0.99	0.72	0.36	0.27
					Blood glucose			
C.T.	Before	146	5	23	45	22	27	22
	During	99	20	41	16	12	7	4
H.F.	Before	155	25	59	49	18	23	—4
	During	95	39	16	12	21	23	7
R.B.	Before	249	10	34	9	24	10	—14
	During	120	18	41	22	16	21	17
A.J.	Before	238	41	55	48	36	25	12
	During	137	20	20	40	29	19	6
O.G.	Before	160	—1	55	63	51	49	32
	During	119	32	38	32	27	21	10
Mean	Before	190	16	45	43	30	27	10
	During	114	26	31	24	21	18	9
					Blood fructose			
C.T.	Before	0	71	82	30	14	8	5
	During	1	53	63	23	9	5	2
H.F.	Before	2	63	80	29	14	8	5
	During	2	67	86	33	12	4	4
R.B.	Before	2	70	84	26	16	10	6
	During	1	75	82	25	10	5	4
A.J.	Before	1	82	98	32	17	7	5
	During	1	94	116	32	14	9	4
O.G.	Before	0	95	116	40	18	10	6
	During	1	86	108	29	14	7	5
Mean	Before	1	76	92	32	16	8	6
	During	1	75	91	28	12	6	4

*One gram of fructose per kilogram was administered intravenously in one hour.
†Unless indicated otherwise, values are positive.

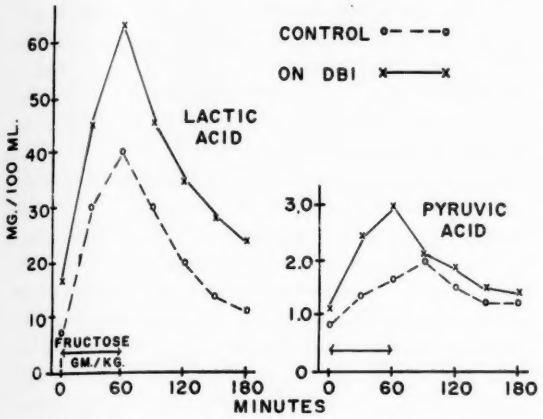

FIG. 3. Influence of DBI on the average changes in the blood concentrations of lactic acid and pyruvic acid produced by fructose administration in five diabetic patients (C.T., H.F., R.B., A.J., and O.G.). The pyruvic acid concentrations are plotted on a scale ten times as great as the lactic acid scale.

32.6 mg. per 100 ml. during the control period and 46.1 per 100 ml. during DBI therapy. In four of the five cases the greater elevation of the lactic acid concentration above the fasting level when the subject was receiving DBI persisted for at least two hours after the cessation of fructose administration. In three cases the pyruvic acid rise was greater during DBI therapy, while the rate of decline after cessation of the fructose infusion did not appear to be impaired. The increases in the plasma citric acid concentration were somewhat less during DBI administration in three of the four patients tested.

Lactate tolerance tests (*table 5 and figure 4*): In all six patients tested sodium lactate administration produced a greater rise in the blood lactic acid concentration when the patient was receiving DBI than during the control period; the average maximum rise was 25.6 mg. per 100 ml. during therapy with DBI as compared to an average increase of 21.2 mg. per 100 ml. during the control period. Following the cessation of lactate ad-

TABLE 5

Influence of DBI on the changes produced in the blood concentrations of metabolites by sodium lactate administration* in diabetic patients† (mg. per 100 ml.)

Subject	DBI therapy	Fasting	Change from fasting: Minutes after start of lactate infusion					
			30	60	90	120	150	180
					Blood lactic acid			
C.T.	Before	6.3	13.3	19.4	9.9	6.7	4.8	1.7
	During	14.3	14.6	22.9	14.3	11.4	9.3	5.0
H.F.	Before	13.3	7.1	10.3	6.0	2.1	0	—2.5
	During	19.2	17.5	19.7	5.2	1.2	0.8	—4.4
R.B.	Before	8.1	15.3	20.5	9.4	4.6	1.8	0.1
	During	24.8	8.4	24.1	7.1	0	—1.4	—4.1
A.J.	Before	4.9	14.5	24.1	11.8	5.7	3.6	2.4
	During	23.8	15.1	26.0	12.1	—3.3	1.1	—1.8
O.G.	Before	4.3	19.2	21.6	4.7	1.7	0.5	—0.3
	During	9.6	17.9	25.0	10.7	6.4	2.3	1.5
H.M.	Before	7.2	26.3	31.5	11.3	3.9	2.0	0.1
	During	13.9	16.3	35.9	20.8	11.8	7.3	2.1
Mean	Before	7.4	16.0	21.2	8.8	4.1	2.1	0.2
	During	17.6	15.0	25.6	11.7	4.6	3.2	—0.3
					Blood pyruvic acid			
C.T.	Before	0.88	0.36	0.67	0.80	0.51	0.19	0.24
	During	1.10	0.85	1.09	0.61	0.18	0.29	0.32
H.F.	Before	0.53	0.22	0.30	0.19	0.17	0.19	0.27
	During	0.72	—0.19	—0.02	—0.19	—0.08	—0.05	0.14
R.B.	Before	0.67	0.53	0.40	—0.19	—0.14	—0.24	—0.22
	During	0.59	0.29	0.29	0.11	0.11	0.08	0.08
A.J.	Before	0.80	0.03	0.19	0.64	0.32	0.43	0.38
	During	1.82	0.80	0.64	1.26	—0.19	0.64	0.51
O.G.	Before	1.23	—0.43	—0.37	0	—0.29	—0.29	0
	During	1.12	0.64	0.91	—0.05	0.11	0.08	0.16
H.M.	Before	0.75	0.03	1.15	0.67	0.32	—0.19	0.32
	During	1.23	0.70	0.64	0.78	0.37	0.16	0.21
Mean	Before	0.81	0.12	0.39	0.35	0.15	0.02	0.16
	During	1.10	0.52	0.59	0.42	0.08	0.20	0.24

*1.36 mM of sodium lactate per kilogram was administered intravenously in one hour except to subject H.M. who received double this dose.
†Unless indicated otherwise, values are positive.

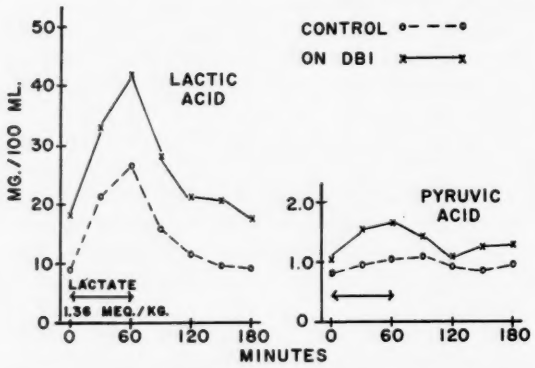

FIG. 4. Influence of DBI on the average changes in the blood concentrations of lactic acid and pyruvic acid produced by sodium lactate administration in five diabetic patients (C.T., H.F., R.B., A.J., and O.G.). The pyruvic acid concentrations are plotted on a scale ten times as great as the lactic acid scale.

ministration, the time required for the blood lactic acid concentration to return to the initial fasting level was longer when the patient was receiving DBI than during the control test in three of the six subjects. In the same three cases, the fractional rate of disappearance of the blood lactate remaining in excess of the fasting level was decreased during DBI administration. The blood pyruvic acid increase produced by lactate administration was greater during DBI administration in three of six subjects. In two of four cases the increase in the plasma citric acid concentration was less when the patient was receiving DBI.

In summary, the daily administration of 150 to 175 mg. of DBI to diabetic patients produced an elevation of the fasting blood lactic acid concentration, an increased daily urinary excretion of lactic acid, a greater rise in the blood lactic acid concentration after fructose administration, and a slightly greater increase in the blood lactic acid concentration after sodium lactate administration. Under the same circumstances, the increases in blood and urinary pyruvic acid were proportionately less than the lactic acid changes.

DISCUSSION

An elevation of the blood lactic acid concentration has been described by other investigators in experimental animals[1] and patients[9,10] who were given DBI. This finding, the increased urinary excretion of lactic acid, and the greater rise in blood lactic acid after fructose administration are consistent with an augmented rate of lactate production. Such an effect of DBI has been observed in tissues in vitro.[1]

The greater blood lactic acid elevation after lactate administration in DBI-treated subjects suggests that there may also be interference with lactate removal. Moorhouse et al.[10] have concluded from the results of pyruvate tolerance tests in diabetic patients that DBI slows the rate of removal of pyruvate.

The observation that the changes in lactate are more marked than those in pyruvate is consistent with an alteration in the lactate:pyruvate ratio in favor of lactate. Such a change implies an increase in the available reduced DPN relative to the oxidized form of the nucleotide, and might occur if oxidative processes were disturbed.[2] It is of interest, therefore, that several investigators have postulated that DBI interferes with oxidative metabolism between succinate and cytochrome c,[11] at the level of cytochrome oxidase,[12] or in the coupling of oxidation and phosphorylation.[13]

It is not clear whether the lactate changes produced by DBI are an integral part of its hypoglycemic action or merely a metabolic side effect. The production of small degrees of blood sugar-lowering by doses of DBI which were too small to cause detectable changes in lactate metabolism in the patients described in this study does not constitute adequate evidence on this point because of the relative insensitivity of the methods employed for the evaluation of lactate metabolism. The increased lactate losses in the urine fell far short of equivalence to the decreases in glycosuria. This finding does not exclude the association of increased glucose removal with increased lactate production, since the lactate formed in one organ might be utilized to a large extent in another organ and thus be unavailable for excretion.

In general, these results are consistent with the occurrence of changes in lactate metabolism in patients receiving DBI which are similar to those which have been produced by DBI in tissues in vitro.

SUMMARY

Eight diabetic patients were studied before and during DBI administration; a maximum daily dose of 150 or 175 mg. of the drug was employed. DBI produced an elevation of the fasting blood lactic acid concentration, an increase in the daily urinary excretion of lactic acid, a greater increase in the blood lactic acid level after fructose administration, and a slightly greater blood lactic acid increase after lactate administration. The blood concentrations and urinary excretion of pyruvic acid were increased under the same circumstances, but the changes were proportionately less than in the case of lactic acid. Increases in the plasma concentration and

urinary excretion of citric acid accompanied DBI administration in some of the subjects. These results are consistent with an effect of DBI on glucose and lactate metabolism in diabetic patients similar to that produced by the drug in tissues in vitro, namely an increased uptake of glucose and an increased production of lactic acid.

SUMMARIO IN INTERLINGUA

Le Acidos Lactic, Pyruvic, e Citric in Patientes Diabetic: Le Influentia de Phenformina

Octo patientes diabetic esseva studiate ante e durante le administration de phenformina. Le maxime dose diurne esseva 150 o 175 mg. Phenformina produceva un elevation del concentration sanguinee de acido lactic in stato jejun, un augmento del diurne excretion urinari de acido lactic, un plus marcate augmento del nivello sanguinee de acido lactic post le administration de fructosa, e un levemente plus marcate augmento del nivello sanguinee de acido lactic post le administration de lactato. Le concentration sanguinee e le excretion urinari de acido pyruvic esseva augmentate sub le mesme conditiones, sed iste alterationes esseva proportionalmente minus marcate que in le caso de acido lactic. Augmentos del concentration plasmatic e del excretion urinari de acido citric accompaniava le administration de phenformina in certes del subjectos. Iste resultatos indica que phenformina exerce super le metabolismo de glucosa e lactato in patientes diabetic un effecto simile a illo producite per le droga in tissus in vitro, i.e. un augmento del acceptation de glucosa e un augmento del production de acido lactic.

ACKNOWLEDGMENT

The authors wish to express their appreciation for the assistance of the dietary and nursing personnel on the Metabolic Research Division of the University Hospitals of Cleveland. The following members of the House Staff assisted in the clinical management of the patients studied: Drs. D. R. Clark, T. M. Daniel, F. S. Donaldson, J. F. Kelley, T. C. Lloyd, Jr., J. R. Mollenkopf, and J. Moses.

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Metabolic Effects of Phenethylbiguanide in Normal Subjects and in Diabetic Patients

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Phenethylbiguanide (formamidinyliminourea or DBI) is a recently developed oral nonsulfonylurea blood sugar-lowering agent.[1] It has been reported that its administration produces hypoglycemia in normal animals[1,2] and causes a reduction of hyperglycemia and glycosuria in diabetic animals[1] and diabetic patients.[3,4] Studies related to the mechanism of action of this compound have been reported from work in animals[2,5] and in isolated tissues.[5]

The experiments described in this report were undertaken in an effort: (1) to ascertain the effects of DBI on the metabolism of carbohydrate, protein and electrolytes, and on adrenal function, and (2) to elucidate possible mechanisms of action of DBI in man. With these objectives in mind we have studied the effect of DBI: (a) upon levels of blood glucose under a variety of testing procedures (fasting, glucose tolerance, glucagon, insulin tolerance); (b) upon metabolic balances; and (c) upon intermediary carbohydrate metabolism in healthy subjects and in diabetic patients.*

SUBJECTS AND METHODS

All subjects for whom metabolic balance studies are reported were admitted to the Metabolism Research Laboratory and were maintained on constant diets. Table 1 summarizes the essential data concerning these subjects and their diets.

The other healthy subjects ingested diets containing 250 gm. of carbohydrate or more, and maintenance calories for at least three days before all tests. The other diabetic patients maintained their usual diets. With the exception of K.L., all patients taking insulin discontinued its use throughout the experimental periods. In all subjects DBI was given in the amounts indicated in three or four divided doses with meals.

Chemical analyses performed on blood, serum and urine were determined by standard methods previously used in this laboratory.[8] Venous blood samples for pyruvate[9] and lactate[10] were collected without veno-occlusion after the subjects had been recumbent for at least one-half hour. Glucose tolerance tests, intravenous insulin tolerance tests and glucagon tests were carried out as previously reported.[8] Intravenous pyruvate tolerance tests* were performed by administering 10 gm. of sodium pyruvate as a 10 per cent solution over four minutes.

RESULTS

A. Effect of DBI *on blood glucose levels.*

Fasting blood glucose levels: In ten separate experiments DBI was administered as a single dose of 100 to 400 mg. to three normal fasting subjects. As seen in table 2 there was no decrease in blood glucose levels over a period of four to eight hours whether the subjects had been on a normal diet or after they had eaten a 600-calorie diet for two weeks.

In two of these subjects DBI was given for nine days, and in one of the two for a third course of twelve days. As can be seen from the mean levels during the control, DBI, and recovery periods, the drug had no effect on fasting blood glucose when given in daily dosages up to 250 mg. per day (table 3).

In contrast, administration of DBI caused a fall in the level of the fasting blood glucose in nonketotic stable diabetic patients. In the doses tolerated (up to 250 mg. per day) the blood glucose levels fell to normal values in some but not in all patients (table 4 and figures 1 and 2).

DBI produced no change in the fasting blood glucose level in K.L., a juvenile type of diabetic who was given a constant but suboptimal amount of insulin (table 4). In patient B.S., a tolbutamide-nonresponsive diabetic, withdrawal of 25 units of NPH insulin resulted in a

Presented at the Symposium on "A New Oral Hypoglycemic Agent, Phenformin (DBI)" in Houston on Feb. 5, 1959.

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*Preliminary reports of these studies have appeared in abstract form.[6,7]

*Our statement in an abstract that the "pyruvate tolerance test" is a new procedure is incorrect.[11-13]

TABLE 1

Subjects				Diabetes			Diet				
Initials	Sex	Age	Wt. kg.	Duration	Type	Insulin requirement, units	Nitrogen gm.	Carbohydrate gm.	Calories	Na mEq.	K mEq.
R.R.*	M	23	70	—	—	—	18.9	313	2932	213	115
J.D.*	M	20	69	—	—	—	18.9	313	2932	213	115
K.W.	M	30	61	25	Labile	35	15.4	198	2140	225	120
C.W.	F	61	60	13	Stable	40-60	14.2	168	1760	109	97
R.B.	F	63	69	3	Stable	35	13.4	182	1670	113	61
B.S.†	M	35	60	12	Stable	25	100 Protein	200	2000	—	—

* Healthy subject. † Studied on medical ward of University Hospital.

TABLE 2

Effect of administration of a single dose of DBI on blood sugar in fasting normal subjects

Dosage mg.	Subjects	F	½	1	1½	2	2½	3	3½	4	5	6	7	8
100	D.M.	86	86	78	87	89	89	85	83	81				
100	R.R.	79	84	74	78	80	77	77	76	77				
200	D.M.	67	71	77	77	79	71	71	77	—				
200	R.R.	90	—	92	—	89	—	85	—	94	86	92		
200	J.D.	87	—	86	—	84	—	81	—	85	88	86		
250	R.R.	86	—	81	—	78	—	74	—	76	75	74	71	78
250	J.D.	83	—	79	—	78	—	76	—	79	73	75	75	74
400	R.R.	88	—	85	—	82	—	85	—	84	85	82	—	—
	Mean	83	80	82	81	82	79	79	79	82	81	82	73	76
250*	R.R.	64	—	66	—	77	—	65	—	66	61	60	58	62
250*	J.D.	62	—	60	—	64	—	61	—	63	61	60	60	64
	Mean	63	—	63	—	71	—	63	—	65	61	60	59	63

*After subjects had eaten a 600 calorie (54 P—64 C—16 F) diet for fourteen days.

TABLE 3

Metabolic effects of DBI in normal subjects

				Mean urinary excretion/24 hours						
Subject	Period	Days	Mean FBS mg./24 hr.	Creatinine	Nitrogen	17-OH	17-KS	Na mEq.	Cl mEq.	K mEq.
R.R.	Control	8	81	1.95	18.1*	10.8	21.8	206	230	92
	DBI[1]	12	81	1.84	18.3†	10.3	20.3	195	218	98
	Recovery	9	86	2.01	17.9‡	9.7	23.8	213	230	99
	Control	12	83	2.15	15.9	10.3	20.8	213	226	103
	DBI[2]	9	83	2.07	16.6	13.0	20.8	202	219	100
J.D.	Control	8	80	2.30	12.2	11.6	19.4	189	204	84
	DBI[3]	9	78	2.01	11.9	10.4	25.9	176	191	75
	Recovery	3	80	2.18	12.4	9.7	25.9	178	185	85

[1] Daily dose in mg.: 100, 125, 150, 200 x 6, 250 x 3.
[2] Daily dose in mg.: 150, 200, 200, 250, 175, 200 x 3, 100.
[3] Daily dose in mg.: 150, 200, 200, 250, 175, 200 x 3, 100.

*Includes stool nitrogen of 1.1 gm.
†Includes stool nitrogen of 1.0 gm.
‡Includes stool nitrogen of 1.1 gm.

rise of the fasting blood sugar from normal values to 291 mg./100 ml. in three days. Urinary excretion of glucose was 167 gm. per twenty-four hours on the third day of insulin withdrawal and there was moderate ketonuria. DBI was administered in a dosage of 150 to 200 mg. per day and produced a decrease in hyperglycemia and glycosuria (to 91 gm.) on the third day. On the fourth day of drug therapy 30 units of Regular Insulin were given over twenty hours because of persistent ketonuria. Over the next five days levels of fasting blood glucose varied between 135 and 180 mg./100 ml. and urinary excretion of glucose varied between 2.4 and 29 gm. per day. Moderate ketonuria persisted.

Glucose Tolerance Tests: Glucose tolerance tests were performed in ten healthy subjects before and during administration of DBI. As can be seen in figure 4, DBI did not cause a significant change in blood glu-

TABLE 4
Metabolic effects of DBI in diabetic subjects

Subjects	Period	Days	Mean urinary excretion per 24 hours FBS mg./24-hr.	Creatinine gm.	Glucose gm.	Nitrogen gm.	17-OH mg.	17-KS mg.	Na mEq.	Cl mEq.	K mEq.
K.L.*	Control	5	292	1.52	116	15.9	5.9	13.4	239	243	120
	DBI[1]	11	297	1.49	119	15.8	6.5	14.3	193	213	92
	Recovery	5	281	1.72	191	17.3	5.3	13.7	262	286	115
C.W.	Control	8	306	1.49	9.5	14.1	5.7	13.7	114	116	77
	DBI[2]	14	165	1.42	2.6	14.0	8.3	12.7	99	108	66
	Recovery	5	270	1.38	7.3	14.1	5.9	10.5	99	111	65
R.B.	Control	8	242	1.20	4.2	9.5	4.1	10.2	137	134	61
	DBI[3]	14	88	1.12	1.1	9.2	8.2	10.1	77	95	51
	Recovery	5	176	1.17	1.0	9.3	5.9	10.1	127	127	65

[1] Daily dose in mg.: 100, 175, 200, 250 x 7, 300 x 2.
[2] Daily dose in mg.: 100 x 2, 125, 175, 200, 150, 175, 200, 200, 225, 250 x 3, 100.
[3] Daily dose in mg.: 100, 150, 175 x 8, 100, 125 x 3.
*Maintained on 15 units PZI throughout study.

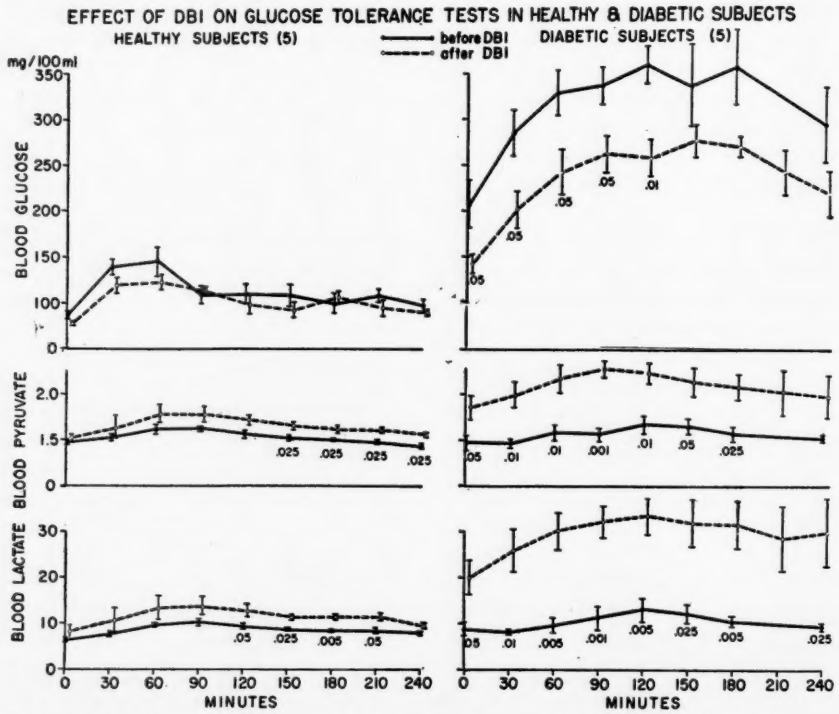

FIG. 1. Effect of DBI on blood levels of glucose, pyruvate and lactate during glucose tolerance tests in healthy subjects and in diabetic patients. The perpendicular bars indicate the standard errors of the means. Where the difference between the means was statistically significant, the *p* value is recorded directly below the two points.

cose levels during the glucose tolerance tests.

Glucose tolerance tests were performed before and during administration of DBI in five diabetic subjects. Although the fasting blood glucose level was lowered significantly by administration of the drug, there was no decrease in the height or duration of the hyperglycemic response after glucose (figure 1).

Glucagon tests: Glucagon tests were performed in three healthy subjects seven times before, and six times after administration of DBI. As can be seen from figure 5, the hyperglycemic response to glucagon was the same or slightly greater after administration of DBI. The somewhat greater response to glucagon was statistically significant only thirty minutes after injection

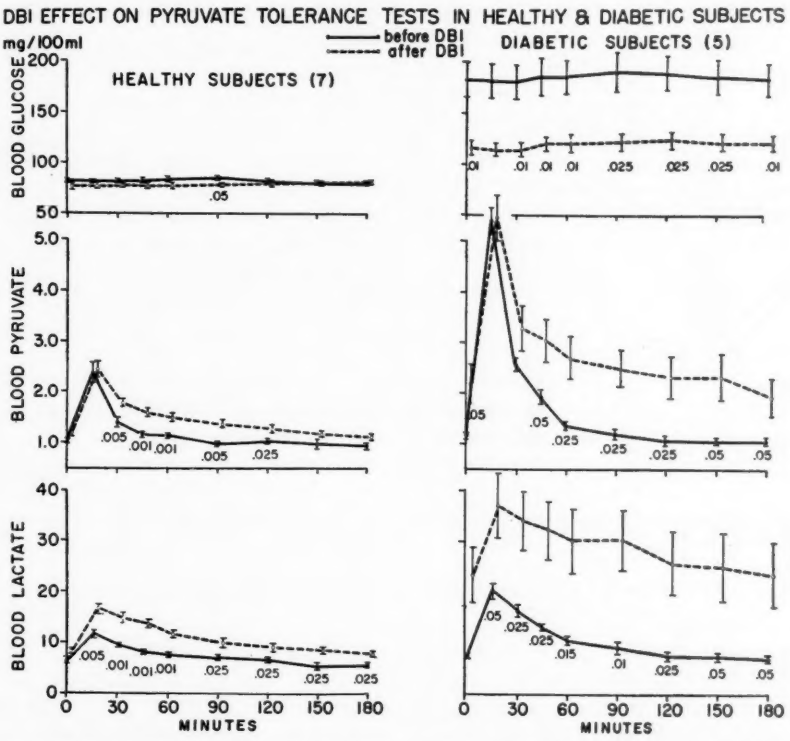

FIG. 2. Effect of DBI on blood levels of glucose, pyruvate and lactate during pyruvate tolerance tests in healthy subjects and in diabetic patients. The perpendicular bars indicate the standard errors of the means. Where the difference between the means was statistically significant, the p value is recorded directly below the two points.

of glucagon ($P<0.05$).

Glucagon tests were performed in two stable diabetic subjects. The hyperglycemic response to glucagon was slightly greater (88 mg. to 199 mg./100 ml.) during DBI therapy than before its administration (242 mg. to 319 mg./100 ml.) in one subject, and was the same in the other patient.

Intravenous insulin tolerance tests: In two healthy subjects intravenous insulin tolerance tests were performed six times as indicated in figure 3. The administration of DBI did not alter insulin sensitivity on one occasion (R.R.), decreased it slightly on another occasion (J.D.) and caused a definite decrease in insulin sensitivity on two other occasions (R.R.). Urinary excretion of 17-hydroxycorticoids was slightly elevated (12.7 mg./24 hrs.) immediately preceding one of these days.

Insulin tolerance tests (0.05 units/kg.) were performed before and during administration of DBI in two diabetic subjects. DBI produced no change in insulin sensitivity although the fasting blood sugar had decreased from hyperglycemic to normal levels.

B. Effect of DBI *on metabolic balances and upon renal excretion of steroids.*

Three metabolic balance studies were conducted in two healthy subjects (table 3). In these three experiments administration of DBI caused no significant changes from control averages for the mean urinary excretion of nitrogen, 17-ketosteroids and potassium. Excretion of creatinine, sodium and chloride was slightly lower during administration of DBI. There were no changes in serum levels of sodium, potassium, chloride, creatinine, blood urea, and hematocrit. On one occasion mean urinary excretion of 17-hydroxycorticoids was higher during administration of DBI (R.R.).

Metabolic balance studies were performed on three diabetic subjects (table 4) to investigate the relationship of nitrogen excretion to carbohydrate metabolism and to determine whether a decrease in gluconeogenesis from protein could be one of the causes of decreased

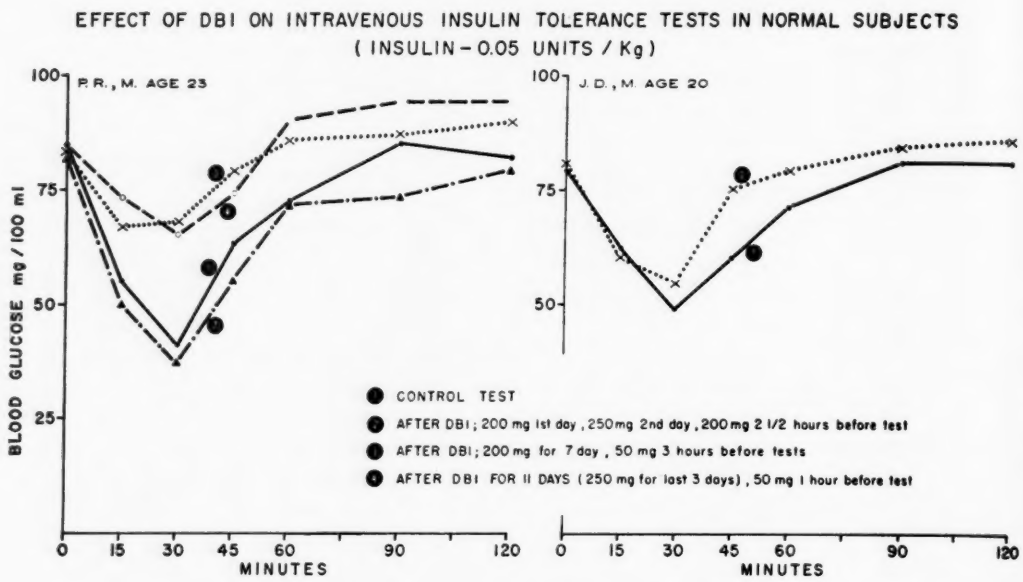
EFFECT OF DBI ON INTRAVENOUS INSULIN TOLERANCE TESTS IN NORMAL SUBJECTS
(INSULIN – 0.05 UNITS / Kg)
P.R., M. AGE 23
J.D., M. AGE 20
BLOOD GLUCOSE mg / 100 ml
100
75
50
25
0 15 30 45 60 90 120
MINUTES
CONTROL TEST
AFTER DBI; 200 mg 1st day, 250mg 2nd day, 200mg 2 1/2 hours before test
AFTER DBI; 200 mg for 7 day, 50 mg 3 hours before tests
AFTER DBI FOR 11 DAYS (250 mg for last 3 days), 50 mg 1 hour before test

FIG. 3. Effect of DBI on intravenous insulin tolerance test (0.05 units/kg.) in normal subjects.

hyperglycemia and glycosuria following administration of DBI. In subject K.L. mean urinary excretion of glucose and nitrogen was the same during an eleven-day period of DBI administration as during the control period. However, on several days during the second half of the DBI period, glycosuria was significantly diminished. Urinary nitrogen excretion fluctuated in the same direction as glucose excretion. Following discontinuation of DBI there was a progressive increase in glycosuria and an increase in nitrogen excretion. Although it is impossible to be certain of any effect of DBI in this patient, one gains the impression that the drug may have prevented the further intensification of the diabetic state which followed the withdrawal of the drug.

Since in grossly uncontrolled diabetic patients, large, daily, spontaneous fluctuations occur in the quantities of glucose and nitrogen excreted, patients with minimal glycosuria were chosen as subjects for further balance studies. In subject C.W., DBI produced a fall in the fasting blood sugar from 306 to 165 mg./100 ml. As can be seen from table 4 there was no change in urinary nitrogen excretion during the DBI period. In subject R.B. the level of fasting blood sugar fell from 242 to 88 mg./100 ml. while the patient was given DBI. Again there was no significant change in nitrogen excretion.

During administration of DBI to the three patients on balance study the following were noted: (1) urinary excretion of 17-hydroxycorticoids rose slightly in all, and excretion of 17-ketosteroids remained unchanged; (2) urinary excretion of creatinine remained essentially unchanged but increased in subject K.L. in the recovery period; (3) excretion of sodium and chloride was definitely decreased in subjects K.L. and R.B., and potassium excretion was decreased in subject K.L. and possibly in subject R.B.; and (4) there were no significant changes in serum sodium, chloride, potassium, hematocrit and blood urea nitrogen.

C. Effect of DBI *upon the metabolism of pyruvate and lactate.*

Blood levels of pyruvate and lactate were measured in the fasting state, during oral glucose tolerance tests and during intravenous pyruvate tolerance tests in normal subjects and in diabetic patients both before and during the administration of DBI. Each glucose tolerance test or pyruvate tolerance test was performed on the same subject before and after administration of 200 mg. of DBI daily for at least two days, an additional dose of 50 mg. being given two to three hours before the beginning of the second test.

In normal subjects DBI caused no change in the fasting levels of pyruvate or lactate. DBI did produce slight increases in blood pyruvate and lactate levels during the glucose tolerance tests and slight but highly significant elevations of these levels during the pyruvate

tolerance tests (figures 1 and 2). The blood glucose levels were not changed by administration of DBI to normal subjects.

In the diabetic subjects DBI caused marked increases in the blood levels of pyruvate and lactate both during fasting and during the glucose tolerance and pyruvate tolerance tests (figures 1 and 2). In these subjects DBI produced marked depression of blood glucose levels. The blood levels of pyruvate and lactate during the first sixty minutes of the pyruvate tolerance tests, before DBI was given, were higher in the diabetic than in the normal subjects (figure 2).

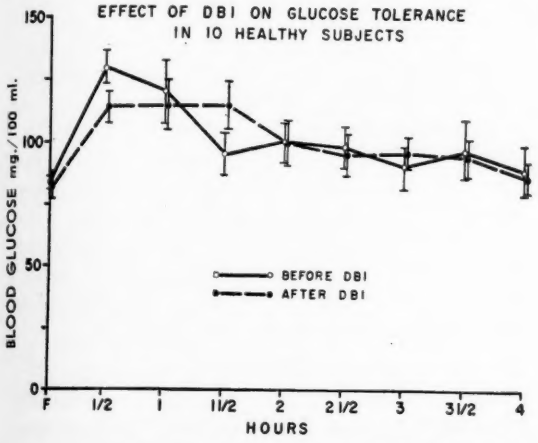

FIG. 4. Effect of DBI on glucose tolerance in ten healthy subjects. DBI was given in a dosage of 200 mg. per day for two days in six experiments, and 300 mg. per day for two days in two experiments. In addition the subjects received 50 to 200 mg. of DBI one to three hours before the second glucose tolerance test.

Urinary lactate[10] was determined in two subjects. In subject R.B. maximum urinary excretion of lactate during the DBI period was 2.86 gm. per 24 hours (0.19 during control period). Blood lactate rose from 7.4 mg./100 ml. in the control to 35.8 mg./100 ml. in the DBI period. In subject B.S., in whom DBI had produced a decrease in glycosuria from 167 gm. to between 29 and 2.5 gm. per day, maximal urinary excretion of lactate was 12.5 gm. per twenty-four hours on the day of minimum glycosuria.

DISCUSSION

As has been reported by others[3,4] DBI produces marked lowering of the blood glucose level in some diabetic subjects. However, in contrast to the findings in normal animals[1] DBI has no effect on blood glucose of healthy human subjects. There was no significant effect on blood glucose levels up to eight hours after

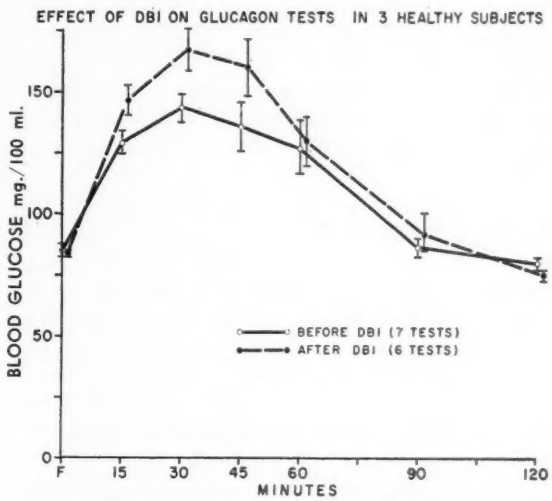

FIG. 5. Effect of DBI on glucagon tests in three healthy subjects. DBI was given in a dose of 200 mg. per day for two days in one experiment and for five to seven days in the other five experiments.

administration of a single dose of DBI in fasting healthy subjects. The dosage used extended up to 5.7 mg./kg. of body weight. In monkeys a single dose of 5 mg./kg. produced hypoglycemia three to five hours after its administration.[1] Even when these subjects had ingested a diet containing 64 gm. of carbohydrate and 600 calories for two weeks DBI produced no measurable hypoglycemic effect. Also, DBI had no effect on fasting blood glucose levels when it was administered for periods up to twelve days.

The data indicate that DBI did not interfere with the hyperglycemic action of glucagon in healthy or in diabetic subjects. This suggests that DBI does not lower blood glucose by decreasing the rate of any of the three enzyme systems (phosphorylase, phosphoglucomutase, or glucose-6-phosphatase) involved in hepatic glycogenolysis. These findings differ from those reported in DBI-treated animals in which glucagon did not increase hepatic glucose output.[2,5]

In contrast to a previous report[3] DBI had no significant effect on glucose tolerance in healthy or in diabetic subjects. DBI did not alter sensitivity to insulin in the normal or in the diabetic subjects when evaluated by the intravenous insulin tolerance test. This suggests that DBI does not exert its hypoglycemic effect by potentiation of insulin activity (endogenous or exogenous). The same conclusion has been reached by a different technic in animal experiments.[2,5]

Metabolic balance studies in the diabetic patients

showed that decreases in hyperglycemia were not accompanied by decreased excretion of urinary nitrogen. Thus, there is no evidence in man that a decrease in the rate of gluconeogenesis from protein is a contributing factor to the hypoglycemic effect produced by DBI. Such a mechanism has been postulated from studies in animals.[5]

The data indicate that decreased function of the pituitary-adrenal system is not one mode of action of DBI. There were no decreases in renal excretion of 17-hydroxycorticoids or 17-ketosteroids and results of metabolic balance studies did not suggest decreased adrenal cortical activity. On the contrary, during several periods of DBI administration there were increases in the mean excretions of 17-hydroxycorticoids. This may have been related to the anorexia and nausea produced by administration of DBI in these subjects.

The data discussed so far do not reveal the mechanisms by which DBI lowers the concentration of blood glucose in diabetic subjects. They do help to eliminate possibilities which had to be considered.

DBI produces an increase in blood pyruvate and lactate levels during pyruvate tolerance tests as well as during glucose tolerance tests in both healthy and diabetic subjects. This suggests that DBI causes a delay in the utilization of pyruvate. In the diabetic subjects the marked elevations in blood pyruvate and lactate levels were associated with sharp decreases in blood glucose levels. In the healthy subjects, the small increases in blood pyruvate and lactate were not accompanied by changes in blood glucose levels. These data suggest that the hypoglycemic effect of DBI may be quantitatively related to and the result of its effect on pyruvate disposal.

Interpretation of the data in this way can be supported and extended by reference to the work of others upon the effects of DBI in isolated tissues.[14–16] It has been shown that DBI inhibits one or more reactions within the Krebs oxidative cycle, thereby diminishing the uptake of oxygen, and enhancing the breakdown of glucose by anaerobic glycolysis to form pyruvate and lactate. Increased utilization of glucose as a result of decreased utilization of oxygen is known as the Pasteur effect.[17] Reduction of the blood glucose level by such a mechanism in the intact animal or human subject has not been recognized. Nevertheless, our experiments are consistent with the hypothesis that DBI produces its hypoglycemic effect in diabetic patients by inhibiting cellular oxidative reactions, thereby increasing the utilization of glucose by anaerobic metabolism. Further support for this hypothesis is found in the recent report that acetylsalicylic acid and dinitrophenol, agents which uncouple oxidative phosphorylation, also have a hypoglycemic effect in diabetic subjects.[18]

It must be pointed out that our data give no indication of the fate of the DBI-induced accumulation of pyruvate other than that some of it is found in the urine as lactate. Anaerobic degradation of glucose to lactate is an energy-yielding reaction of relatively low magnitude. It must be emphasized that a given decrease of blood sugar induced by DBI does not have the same metabolic implications as one induced by insulin.

The reason for the much greater effect of DBI upon blood pyruvate and lactate levels in the diabetic as compared to the healthy subjects is not apparent from present knowledge. The marked difference suggests that there may have been a pre-existing endogenous block in pyruvate metabolism in the diabetic subjects. The presence of such a biochemical lesion in diabetes has been suspected in the past, but it has not been clearly defined.[19–23] The elevation of the first part of the pyruvate tolerance curve in the diabetic subjects before they were given DBI may also be a reflection of an impairment of pyruvate disposal, but such an interpretation is not warranted on the basis of the present data.

SUMMARY

Administration of phenformin to diabetic subjects produces lowering of the blood glucose level. In sharp contrast to the findings in normal animals, phenformin has no effect on blood glucose in healthy people. Phenformin causes no changes in glucose tolerance or sensitivity to insulin, has no effect on glucagon-induced hyperglycemia, and does not decrease urinary excretion of nitrogen, potassium, 17-hydroxycorticoids and 17-ketosteroids in healthy subjects or in diabetic patients. Phenformin increases blood pyruvate and lactate levels during glucose tolerance tests and during pyruvate tolerance tests. This effect is much greater in diabetic patients than in normal subjects.

Thus, there is no evidence that inhibition of adrenal function, decreased gluconeogenesis from protein, increased insulin activity or decreased glucagon activity are contributing factors to the blood glucose-lowering effect produced by phenformin. The findings suggest that phenformin delays the disappearance of pyruvate and lactate from blood. Comparison of the data from normal and diabetic subjects indicates that the hypoglycemic action of phenformin may be quantitatively related to the effect on pyruvate disposal. These results, together with certain in vitro studies by other workers,

suggest that phenformin produces its hypoglycemic effect by causing an increase in anaerobic glycolysis as a result of suppression of cellular oxidation.

SUMMARIO IN INTERLINGUA

Effectos Metabolic de Phenethylbiguanida (DBI) *in Subjectos Normal e in Patientes con Diabete*

Le administration de DBI a subjectos diabetic produce un reduction del nivello sanguinee de glucosa. Per contrasto marcate con le constatationes in normal animales, DBI ha nulle effecto in le glucosa del sanguine de humanos normal. DBI causa nulle alteration del tolerantia pro glucosa o del sensibilitate pro insulina, ha nulle effecto super le hyperglycemia inducite per glucagon, e non augmenta le excretion urinari de nitrogeno, kalium, 17-hydroxycorticoides, e 17-cetosteroides in subjectos normal o in patientes con diabete. DBI augmenta le nivellos de pyruvato e lactato durante tests de tolerantia pro glucosa e durante tests de tolerantia pro pyruvato. Iste effecto es multo plus grande in patientes diabetic que in subjectos normal.

Assi il existe nulle supporto pro le assertion que le inhibition del function adrenal, un reducite gluconeogenese ab proteina, un augmento del activitate de insulina, o un reduction del activitate de glucagon es factores contribuente al effecto glucoso-reductori de DBI. Le constatationes suggere que DBI retarda le disparition de pyruvato e de lactato ab le sanguine. Le comparation de datos ab subjectos normal e diabetic indica que le action hypoglycemic de DBI es forsan relationate quantitativemente al effecto super le elimination de pyruvato. Iste observationes—insimul con le resultatos de certe studios in vitro per altere investigatores—suggere que DBI produce su effecto hypoglycemic per causar un augmento del glycolyse anaerobie in consequentia del suppression de oxydation cellular.

ACKNOWLEDGMENT

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Effect of Phenformin on Peripheral Glucose Utilization in Human Diabetic and Nondiabetic Subjects

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Neither the precise biochemical mechanisms whereby phenethylbiguanide (DBI) lowers blood glucose concentration in human subjects nor its site of action, whether in the peripheral tissues and/or the liver, are known. In the absence of a urinary loss of glucose or glucose intermediaries, hypoglycemia can result only from an increase in peripheral glucose utilization or a decrease in hepatic output of glucose. Although many factors may be involved in either increased peripheral utilization or decreased hepatic production of glucose, the localization of the site of action of DBI might offer some insight into its possible mechanism of action.

The present study was designed to determine the effect of DBI on peripheral glucose utilization in diabetic and normal human subjects. Changes in peripheral glucose utilization* were measured by following the changes in femoral arteriovenous glucose concentration difference after the administration of DBI.

METHODS

Fourteen normal and eleven diabetic subjects were studied. Blood glucose concentration was measured in all twenty-five subjects after the administration of DBI and changes in arteriovenous glucose difference were followed in five of the normal and seven of the diabetic subjects. The studies were performed in a constant temperature room with the subjects in the postabsorptive state. All the diabetic subjects were of the "maturity onset" or stable type and were controlled by diet alone or by diet plus tolbutamide, in which case the sulfonylurea therapy was stopped forty-eight to seventy-two hours prior to each experiment.

Blood samples were collected simultaneously from the femoral artery and the contralateral femoral vein by means of indwelling Cournand needles. After collecting two paired control blood samples, 150 to 350 mg. of DBI were given orally over a one- to two-hour period. Immediately after starting DBI administration blood samples were collected at twenty-minute intervals for five hours in the diabetic subjects; in normal subjects, blood specimens were obtained at thirty-minute intervals from the first to the fourth hour and at twenty-minute intervals for the ensuing two hours.

Glycolysis was minimized by collecting the blood in chilled tubes containing sodium fluoride. Immediately thereafter blood glucose was determined in duplicate by the Somogyi iodometric method[1,2] on 5 ml. of filtrate prepared from 2 ml. blood samples; in our laboratory duplicate determinations checked within 1 mg. per cent, thereby permitting as Somogyi has already indicated[3] the valid measurement of arteriovenous glucose concentration differences of as little as 2 mg. per cent.

RESULTS

Changes in femoral arterial and venous glucose concentrations and in arteriovenous glucose differences following the administration of DBI to normal and diabetic subjects are listed in tables 1 and 2 respectively. Examples of experiments in one normal subject and two diabetic subjects are shown in figures 1-3.

In the fourteen normal subjects, the acute administration of 150-300 mg. of DBI failed to lower blood glucose concentration significantly (table 1). In contrast, the administration of 200-350 mg. of DBI to eleven diabetic subjects produced a decline in blood glucose which was apparent within the first hour (table 2).

In both the five normal subjects and the seven dia-

*Peripheral glucose utilization is used to connote peripheral glucose disappearance which in turn may result from either increased oxidative metabolism, glycogen storage, lactic acid production or conversion to fat.

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TABLE 1

Effect of DBI on arterial and venous glucose concentrations in normal subjects

Subject	Age	Dose DBI mg.		Control 0-15	0	60	90	Time after DBI administration 120	150	180	210	240	260	280	300	320	340	360
K.P.W.	28	225	Art.	87.0	85.6	86.5	85.1	84.5	84.0	82.6	86.2	93.2	88.7	89.0	86.2	82.6	83.4	82.3
59 kg.			Ven.	85.6	84.5	83.7	84.2	84.2	83.1	80.9	84.5	92.9	87.6	89.6	85.1	80.9	82.8	82.3
			A-V	1.4	1.1	2.8	0.9	0.3	0.9	1.7	1.7	0.3	1.1	—0.6	1.1	1.7	0.6	0.0
H.B.	23	200	Art.	87.8	87.3	85.8	86.1	85.3	87.8	84.4	84.1	85.0	86.3	87.2	90.3	91.0	91.5	92.1
82 kg.			Ven.	86.1	83.6	84.1	84.1	82.4	87.4	82.7	82.7	82.7	83.3	88.0	90.6	89.8	88.9	92.1
			A-V	1.7	3.7	1.7	2.0	2.9	0.4	1.7	1.4	2.3	3.0	—0.8	—0.3	1.2	2.6	0.0
B.K.	26	200	Art.	90.1	90.4	87.0	85.7	84.4	84.7	84.1	82.7	83.0	85.3	81.3	79.6	80.4	80.4	80.7
68 kg.			Ven.	87.0	87.3	85.3	83.9	82.4	82.4	83.8	80.4	81.6	83.0	79.9	77.9	79.9	79.0	78.4
			A-V	3.1	3.1	1.7	1.8	2.0	2.3	0.3	2.3	1.4	2.3	1.4	1.7	0.5	1.4	2.3
M.L.	26	175	Art.	83.8	84.4	84.4	84.1	83.8	81.9	80.1	77.3	78.7	78.4	83.6	83.6	82.4	82.7	82.4
60 kg.			Ven.	82.7	83.6	82.4	82.0	81.6	79.3	76.1	75.0	77.8	76.1	82.1	82.1	79.9	79.3	79.6
			A-V	1.1	0.8	2.0	2.1	2.2	2.6	4.0	2.3	0.9	2.3	1.5	1.5	2.5	3.4	2.8
J.H.	25	300	Art.	78.0	78.6	77.6	77.0	78.4	77.0	80.8	76.8	73.0	71.0	68.0	69.2	70.8	70.4	70.0
74 kg.			Ven.	76.8	78.0	76.0	74.0	77.6	76.0	78.8	75.6	71.6	68.4	67.2	68.4	68.0	68.8	68.4
			A-V	1.2	0.6	1.6	3.0	0.8	1.0	2.0	1.2	1.4	2.6	0.8	0.8	2.8	1.6	1.6
N.K.	27	200	Ven.		92.9	91.2		88.9		90.3		86.1			93.7			89.2
J.K.	26	200	Ven.		93.7	93.1		91.2		87.0		82.8			82.8			80.8
M.C.	30	200	Ven.		76.0	74.4		75.6		84.8		75.6			80.4			80.6
C.A.	24	150	Ven.		86.0	83.2		83.6		86.2		88.0			90.0			95.6
P.H.	31	200	Ven.		80.2	83.0		87.0		90.8		91.0			93.8			92.6
L.F.	40	200	Ven.		73.2	80.6		78.0		85.4		75.2			83.0			84.0
C.R.	38	150	Ven.		85.0	82.4		84.4		83.4		84.6			83.4			89.6
J.A.	27	150	Ven.		58.0	68.0		68.0		59.6		60.2			59.0			70.8
G.C.	35	200	Ven.		83.6	83.6		89.6		74.0		89.2			92.0			88.0

TABLE 2

Effect of DBI on arterial and venous glucose concentrations in diabetic subjects

Subject	Age	Dose DBI mg.		Control 0-10	0	20	40	60	80	Time after DBI administration 100	120	140	160	180	200	220	240	260	280	300
M.S.	61	250	Art.	157.3	157.2	157.5	151.0	149.0	141.8	137.2	127.8	126.5	122.3	118.5	112.4	106.2	104.5	102.6	100.7	98.8
84 kg.			Ven.	155.3	155.0	158.4	152.0	145.5	140.8	135.0	131.0	125.5	122.3	119.5	111.8	106.8	104.8	100.3	101.2	98.5
			A-V	2.0	2.2	—0.9	—1.0	3.5	1.0	2.2	—3.2	1.0	0.0	—1.0	0.6	—0.6	—0.3	2.3	—0.5	0.3
F.L.	60	300	Art.	162.4	163.4	159.0	154.4	150.2	146.4	140.6	136.0	131.0	127.0	124.0	119.3	114.4	110.6	106.0	100.2	96.0
81 kg.			Ven.	160.1	161.1	158.4	154.0	150.6	145.1	138.9	136.0	129.0	125.0	122.2	118.0	114.4	108.2	104.8	101.0	96.0
			A-V	2.3	2.3	0.6	0.4	—0.4	1.3	1.7	0.0	2.0	2.0	1.8	1.3	0.0	2.4	1.2	—0.8	0.0
M.F.	43	350	Art.	138.1	136.4	135.0	128.2	120.1	117.7	113.9	108.3	103.4	97.1	92.5	85.2	79.6	74.0	73.2	71.0	70.0
67 kg.			Ven.	136.6	133.8	134.0	126.1	118.9	118.0	110.9	105.8	102.2	96.8	90.6	81.4	76.9	72.0	72.0	68.4	68.0
			A-V	1.5	2.6	1.0	2.1	1.2	—0.3	3.0	2.5	1.2	0.3	1.9	3.8	2.7	2.0	1.2	2.6	2.0
B.D.	65	300	Art.	250.0	252.0	255.0	249.0	242.0	236.0	234.0	234.0	224.0	212.0	209.0	204.0					
73 kg.			Ven.	250.0	252.0	254.0	247.0	240.0	236.0	233.0	234.0	223.0	212.0	209.0	203.0					
			A-V	0.0	0.0	1.0	2.0	2.0	0.0	1.0	0.0	1.0	0.0	0.0	1.0					
V.B.	52	300	Art.	198.6	197.6	194.9	191.8	184.5	177.0	169.8	165.9	165.2	165.4	163.7	158.6	158.8	156.4	155.4	151.3	152.3
60 kg.			Ven.	194.7	197.1	195.2	188.1	182.6	177.0	171.8	165.7	162.3	161.3	160.0	157.1	159.8	155.4	157.4	152.0	149.1
			A-V	3.9	0.5	—0.3	3.7	1.9	0.0	—2.0	0.2	2.9	4.3	3.7	1.5	—1.0	1.0	—2.0	—0.7	3.2
D.J.	44	350	Art.	133.0	132.7	129.8	128.4	120.2	116.4	109.2	108.4	108.7	105.2	104.4	98.5	98.5	97.5	92.6	91.0	89.4
72 kg.			Ven.	132.2	131.4	127.6	124.7	119.1	116.7	109.5	107.9	108.4	104.9	101.5	99.1	98.5	96.1	92.6	92.4	89.2
			A-V	0.8	1.3	2.2	3.7	1.1	—0.3	—0.3	0.5	0.3	0.3	2.9	—0.6	0.0	1.4	0.0	—1.4	0.2
J.S.	58	300	Art.	102.0	98.5	98.0	94.4	87.6	84.6	83.0	84.9	80.4	80.1	80.4	77.7	75.3	75.0	74.5	74.5	76.1
70 kg.			Ven.	100.9	97.5	96.4	93.6	89.2	86.0	84.6	83.8	78.8	77.4	76.6	76.4	75.0	73.7	73.4	71.8	72.4
			A-V	1.1	1.0	1.6	0.8	—1.6	—1.4	—1.6	1.1	1.6	2.7	3.8	1.3	0.3	1.3	1.1	2.7	3.7
G.C.	47	200	Ven.		165			149			137			122			110			97
M.D.	58	200	Ven.		132			128			113			110			97			97
H.F.	35	200	Ven.		283			291			274			266			266			262
T.E.	40	250	Ven.		138			118			107			93			74			73

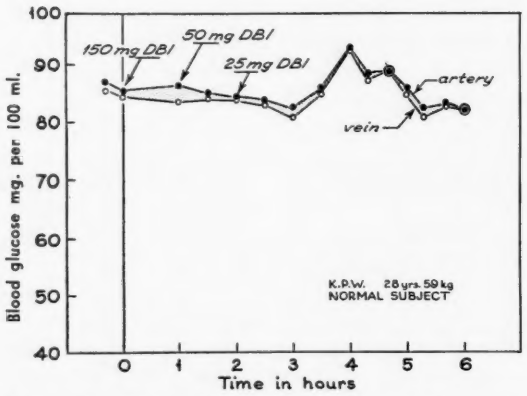

FIG. 1. The effect of DBI on arterial and venous glucose concentrations in a normal subject.

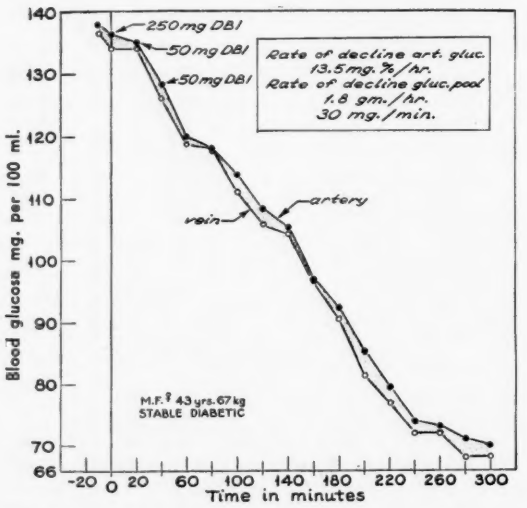
250 mg DBI
50 mg DBI
50 mg DBI
Rate of decline art. gluc. 13.5 mg.%/hr.
Rate of decline gluc. pool 1.8 gm./hr. 30 mg./min.
artery
vein
M.F. ♀ 43 yrs. 67 kg
STABLE DIABETIC
Blood glucose mg. per 100 ml.
Time in minutes

FIG. 2. The effect of DBI on arterial and venous glucose concentrations in a diabetic subject.

betic subjects, arteriovenous glucose difference did not increase, despite the fall in arterial blood glucose in the diabetic subjects (tables 1 and 2, figures 1-4). Arteriovenous glucose difference during the control periods averaged 1.8 mg. per cent in the normal subjects and 1.7 mg. per cent in the diabetic subjects; after DBI administration arteriovenous glucose difference averaged 1.6 mg. per cent and 1.0 mg. per cent for the normal and diabetic subjects respectively (figure 4).

DISCUSSION

The failure of DBI to lower the blood glucose concentration in *normal human subjects* contrasts sharply

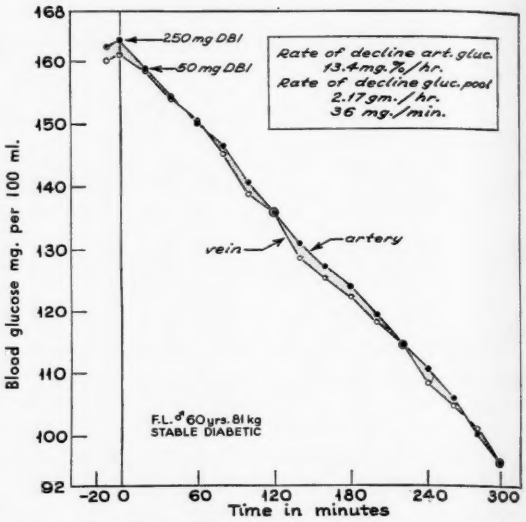
250 mg DBI
50 mg DBI
Rate of decline art. gluc. 13.4 mg.%/hr.
Rate of decline gluc. pool 2.17 gm./hr. 36 mg./min.
vein
artery
F.L. ♂ 60 yrs. 81 kg
STABLE DIABETIC
Blood glucose mg. per 100 ml.
Time in minutes

FIG. 3. The effect of DBI on arterial and venous glucose concentrations in a diabetic subject.

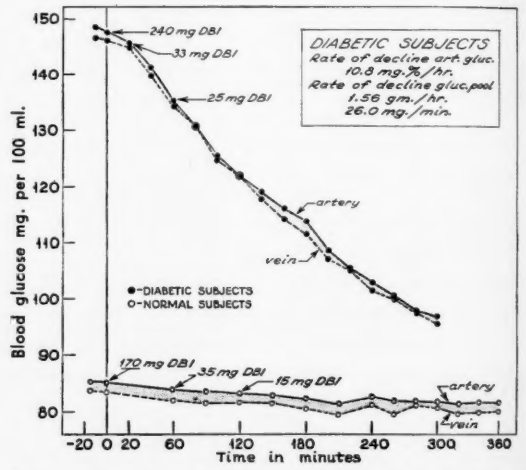

FIG. 4. Comparison of the effects of DBI on mean arterial and venous glucose concentrations in seven diabetic and five normal subjects.

with its effect in nondiabetic laboratory animals of several species including the rat, cat, guinea pig, rabbit, and monkey.[4] Although the dose administered to nondiabetic human subjects (> 3 mg./kg.) was less than that given to nondiabetic animals (5-20 mg./kg.),[4] this dose, nevertheless, reduced the blood glucose of diabetic human subjects. This difference in blood glucose response between nondiabetic human subjects and nondiabetic laboratory animals suggests that the biochemical changes[5-7] following administration of DBI,

which are said to be causally linked with the hypoglycemic effect in laboratory animals, may not be operative in human diabetic subjects.

The failure of DBI to lower blood glucose concentration in nondiabetic human subjects is of considerable interest but is not explained by these data. Although it may be related to the initial level of the blood glucose, it should be noted that a hypoglycemic effect occurred in diabetic subjects with normal and near normal fasting blood glucose concentrations (subjects J.S., M.F., D.J., table 2). Alternatively, the blood glucose lowering effect of DBI in diabetic but not in nondiabetic subjects suggests a specific metabolic defect in the diabetic group which is altered by DBI.

The failure to find any increase in femoral arteriovenous glucose difference associated with the fall in blood glucose concentration in the subjects with stable diabetes indicates that the hypoglycemic effect was either not the consequence of increased peripheral glucose utilization or that the rate of increased peripheral glucose utilization was too small to be measured by the technics of this study. The latter possibility seems unlikely in view of the magnitude of glucose utilization by muscle in human subjects in the basal state compared to the magnitude of decline in the glucose pool in the present experiments.

The rate of decline in the glucose pool, assuming that the glucose space is limited to the volume of distribution of the extracellular fluid, is shown in table 3. The decline in the glucose pool varied from 11.3 to 36 mg. per minute and averaged 26 mg. per minute. Despite the fact that the muscle mass constitutes approximately 40 per cent of body weight, in the basal state only about 22 mg. of glucose are utilized per minute by the entire muscle mass.[8] This rate of utilization by the muscles in the resting postabsorptive state is associated with a mean femoral arteriovenous glucose difference of about 1.8 mg. per cent in this and other studies.[8-10] If the mean rate of decline in glucose pool of 26 mg. per minute, found in this study after DBI (table 3), was entirely the result of increased glucose utilization by the muscles, a doubling of the femoral arteriovenous glucose difference would have been expected provided there was no alteration in peripheral blood flow.

It is possible, however, that an increase in peripheral glucose utilization occurred but because it was confined for the most part to the adipose tissue rather than to muscle, could not be measured by the technic used in this study; blood specimens were collected from the femoral rather than the saphenous vein in these experiments. They therefore most likely reflected changes in muscle rather than in adipose tissue metabolism.

TABLE 3

Effect of DBI on rate of glucose disappearance in diabetic subjects

Subject	Wt. kg.	Glucose pool liters	Fall blood glucose mg./100 ml./hr.	Decrease in glucose pool gm./hr.	Decrease in glucose pool mg./min.
M.S.	84	16.8	11.7	1.90	31.6
F.L.	81	16.2	13.4	2.17	36.1
M.F.	67	13.4	13.5	1.80	30.0
B.D.	73	14.6	14.0	2.04	34.0
V.B.	60	12.0	9.2	1.10	18.3
D.J.	72	14.4	8.7	1.25	20.9
J.S.	70	14.0	4.8	0.68	11.3
Mean	72	14.5	10.8	1.56	26.0

The in vitro evidence[6,7,11] that DBI lowers blood glucose primarily by decreasing oxidative metabolism, and, as a consequence of this, secondarily increasing anaerobic glycolysis is not supported by these data insofar as the peripheral muscles are concerned. The increased utilization of glucose by muscle, whether the result of increased oxidative metabolism, increased glycogen storage or increased conversion to lactate, should have resulted in an increased femoral arteriovenous glucose difference.

The failure in these studies on *human subjects* to ascribe the hypoglycemic effect of DBI to increased glucose utilization by the peripheral muscles, coupled with the failure of Tranquada et al.[12] to obtain evidence of decreased splanchnic glucose output suggests two possible alternate explanations for the hypoglycemic effect. One, the adipose tissue may be a major locus of action of DBI. Two, *more likely* the increase in the rate of glucose utilization of only 26 mg. per minute may have been divided between the liver and the peripheral tissues and therefore would be difficult to detect by measuring changes in either femoral arteriovenous glucose difference or in splanchnic glucose output.

SUMMARY

The changes in arterial blood glucose concentration following the administration of phenformin were followed in fourteen normal and eleven diabetic subjects. The effect of phenformin on peripheral glucose utilization was studied by following changes in femoral arteriovenous glucose difference in five of the normal and seven of the diabetic subjects. Phenformin failed to lower the arterial blood glucose or alter the arteriovenous glucose difference in all the normal subjects. In diabetic subjects despite a fall in mean arterial blood glucose from 148 to 94 mg. per cent, representing a mean decrease in the

glucose pool of 26 mg. per minute, there was no evidence of increased peripheral glucose utilization as manifest by the absence of any increase in femoral arteriovenous glucose concentration difference. The possibility that the hypoglycemic action of phenformin *in diabetic human subjects* was either the consequence of an effect of phenformin on adipose tissue or, more likely, the result of a simultaneous effect on both hepatic and peripheral tissues, was discussed.

SUMMARIO IN INTERLINGUA

Le Effecto de Phenformina Super le Utilisation Peripheric de Glucosa in Humanos Diabetic e Non-diabetic

Le alteration del concentration de glucosa in le sanguine arterial esseva observate post le administration de phenformina (DBI) in dece-quatro subjectos normal e in dece-un diabeticos. Le effecto de DBI super le utilisation peripheric de glucosa esseva studiate per observar alterationes occurrente in le differentia arterio-venose femoral de glucosa in cinque del normales e in septe del diabeticos. DBI non reduceva le glucosa del sanguine arterial e non alterava le differentia arterio-venose de glucosa in ulle del subjectos normal. In le subjectos diabetic — in despecto del reduction del nivello medie de glucosa in sanguine arterial ab 148 a 94 mg pro cento, lo que representa un reduction medie de glucosa in le reserva de 26 mg per minuta — nulle indication esseva trovate de un augmento del utilisation peripheric de glucosa, a judicar per le absentia de omne augmento in le differentia arterio-venose femoral del concentration de glucosa. Le possibilitate es discutite que le effecto hypoglycemic de DBI in patientes diabetic es (1) le consequentia de un effecto de DBI sur tissu adipose o (2), lo que es plus probabile, le resultato de un effecto simultanee sur le tissu hepatic e sur le tissu peripheric.

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Physiological Genetics

The idea that genes control the properties of cells by determining the enzymes that are present goes back in a sense to the earliest years of the century, and the idea that genes act by imposing specific patterns in the process of synthesis of macromolecules, whether in their own duplication, in the production of antigenic specificity, or in that of the enzymes, is also far from recent. These conjectures had, however, little or no real impact on general physiology. Until very recently, the textbooks in that subject rarely made any mention of the gene and treated the cell as the ultimate unit of life.

The real breakthrough came recently when systematic exploration of the control of elementary processes of the mould *Neurospora* was made by the combined efforts of a geneticist and a biochemist, Beadle and Tatum. Now we have a rapid expansion of biochemical genetics or genetic biochemistry. What had been two almost airtight compartments of biology are now inextricably interwoven.

From "Genetics and the Hierarchy of Biochemical Sciences," by Sewall Wright, in *Science,* Vol. 130, No. 3381, pp. 959-65, Oct. 16, 1959.

Some Effects of Phenethylbiguanide on Human Hepatic Metabolism as Measured by Hepatic Vein Catheterization

Robert E. Tranquada, M.D., Charles Kleeman, M.D., and Josiah Brown, M.D., Los Angeles

Phenethylbiguanide (DBI) and its analogues, amyl- and isoamylbiguanide given orally or parenterally, have been shown to have hypoglycemic activity in diabetic humans and several species of normal and alloxan diabetic animals.[1-3] However, the clinical usefulness of the biguanide group of drugs is limited at present both by an incomplete knowledge of their mode of action and by a relatively high incidence of side effects. The fact that DBI is effective in the treatment of the pancreatectomized human diabetic[4] suggests that a more complete understanding of its mode of action might lead to a significant addition to our knowledge of diabetes mellitus and its therapy. Investigations of the mechanism of DBI hypoglycemic activity have included several observations of its effect on animal tissues in vitro and in vivo, as well as a smaller number of observations in the human.

DBI has hypoglycemic activity in alloxan diabetic animals and in the absence of the entire gut, spleen, kidneys, liver, pancreas, and after ligation of the portal vein and hepatic artery, although the latter procedure reduces the hypoglycemic activity of the drug.[5,6] Increased uptake of glucose accompanied by increased lactate production by the rat diaphragm incubated with DBI has been shown by Williams and Tyberghein[6] and oxygen consumption was decreased in a similar preparation.[7,8] On the other hand, Wick has reported a decrease in glucose uptake by the DBI treated rat diaphragm.[9]

Inhibition of gluconeogenesis in the guinea pig liver by DBI has been suggested by the demonstration of decreased incorporation of alanine into glycogen in vivo, by decreased nitrogen excretion by the DBI treated rat[6,7] and by a decreased rate of blood urea nitrogen accumulation in the nephrectomized and DBI treated rat.[7]

Conflicting evidence has been presented concerning the effect of DBI on liver and muscle glycogen, Tyberghein[7] having noted a marked decrease in liver glycogen and a slight decrease in muscle glycogen and Williams[8] a fall in muscle glycogen content in DBI treated preparations, while Ungar and his associates[3] showed a variable response of liver and muscle glycogen to DBI depending on the time elapsed after administration of the drug.

In vivo measurements in the guinea pig of $C^{14}O_2$ production from C^{14}-labeled glucose are not significantly changed by DBI administration,[6] although Wick, using the same criteria, has shown decreased glucose oxidation by DBI treated rat epididymal adipose tissues.[10] Nielsen has observed an apparent decrease in hepatic glucose output in guinea pigs given DBI as measured by direct estimation of hepatic and portal vein glucose content after administration of DBI.[11] Tyberghein has also observed increased lactate formation in the total guinea pig, surviving guinea pig liver slices and in the isolated rat diaphragm after treatment with DBI.[7]

Wick and Larson[10] have demonstrated activity of DBI in blocking the citric acid cycle at the succinic dehydrogenase step and have developed further evidence suggesting a specific role of DBI in interfering with the cytochrome oxidase system. They have suggested that the result of this activity is activation of the Pasteur effect with resultant increased anaerobic glycolysis, decreased oxygen utilization, increased glucose utilization and increased production of lactate and pyruvate by the peripheral tissues. It is postulated that the increased amounts of pyruvate and lactate released in the periphery are metabolized by the liver either through gluconeogenesis or oxidative pathways.

Effects of DBI in the human, other than its known clinical effectiveness in the treatment of diabetes mel-

Presented at the Symposium on "A New Oral Hypoglycemic Agent, Phenformin (DBI)" in Houston on Feb. 5, 1959.

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litus[2,4,11–13] include a decrease in the rate of disappearance from the blood of infused lactate and pyruvate and a significant increase in blood lactate and pyruvate in the fasting, DBI treated human.[14,15]

Because some of the evidence is conflicting, and the greater part derived from animal and in vitro experiments, it was felt that the direct measurement of several of these effects in the totally pancreatic-deficient diabetic human by means of direct hepatic vein catheterization might be of value in the interpretation of the above observations.

METHODS AND MATERIALS

Eight diabetic patients were selected; three had undergone total pancreatectomy for chronic relapsing pancreatitis, three had developed diabetes mellitus during the course of chronic relapsing pancreatitis, and two had labile diabetes requiring relatively high daily insulin dosage. None of the subjects had a family history of diabetes mellitus. In three subjects the study was unsuccessful; in one because of the development of chills and fever during the procedure, one because of clotting of the hepatic vein catheter after two hours of the procedure, and the third because of inability to cannulate the right hepatic vein. Patients were in the postabsorptive state and had not received regular or modified insulin for at least twelve or forty-eight hours, respectively. One-half hour prior to the procedure the patients were given 120 mg. sodium phenobarbital intramuscularly as their only sedation.

Right hepatic vein catheterization was performed with a woven nylon catheter inserted through an antecubital vein under fluoroscopic control. In all cases the catheter was first wedged in the liver and then withdrawn slowly until free flow of blood was permitted and the catheter tip was observed fluoroscopically to be well within the right lobe of the liver. Patients remained supine throughout the entire experiment and no further check of catheter position was made following the initial placement.

Simultaneous hepatic vein and femoral arterial blood specimens were collected at least twice during the initial stabilization period of at least an hour after placement of the catheter, and at one-half hour intervals for four hours following the administration of 150 mg. DBI by mouth. Determinations of glucose, oxygen content and saturation, urea, lactate, pyruvate, and bromsulphalein were performed on each hepatic vein and femoral arterial blood specimen. Just prior to DBI administration six to eight consecutive hepatic vein blood specimens were drawn at the rate of 3 ml. during the first forty-five seconds of each minute for blood sugar determinations as a means of estimating the rapidity and degree of change of the hepatic vein glucose concentration in these patients. Oral temperature and pulse rate were recorded at thirty-minute intervals throughout the procedure.

Blood specimens were collected in heparinized syringes, delivered into heparinized test tubes and a portion of each specimen immediately added to freshly prepared, cold, trichloroacetic acid for lactate and pyruvate determinations. The remainder of the heparinized blood was centrifuged and plasma separated for BSP and urea analysis. Separate specimens in oiled, heparinized syringes were collected for oxygen content and iced prior to determinations performed the same day.

Oxygen saturation and content were measured by the manometric method of Van Slyke and Neill.[16] Glucose was determined in quadruplicate by the Nelson modification of the Somogyi method.[17] Pyruvate and lactate were determined in duplicate by the methods of Friedemann and Haugen[18] and Barker and Summerson,[19] respectively. Urea determinations were by a modification of the method of Karr.[20]

Hepatic blood flow was estimated by the bromsulphalein extraction method as described by Bradley and his co-workers.[21] Much discussion of the reliability of this method has appeared, particularly as related to the significance of results used to compare the hepatic blood flow of one individual or group to that of another.[22–28] However, in the present experiment the information sought was the variation in liver blood flow in each individual rather than an absolute value. By assuming that DBI does not significantly affect the extrahepatic removal of BSP and that neither the constant BSP infusion nor the presence of the catheter significantly affected hepatic blood flow, it was felt that the method was adequate for the present study. Meyers[24] has produced evidence which supports the assumption that the BSP infusion itself does not increase estimated hepatic blood flow. We have determined that the presence of DBI in plasma up to a concentration of 3 mg. per ml. does not influence the spectrophotometric determination of BSP concentration. Plasma volume was estimated at 1.69 liters per square meter of surface area as derived by Murray and his associates.[25]

RESULTS

Results are depicted graphically in figures 1-8. Arterial glucose levels were observed to fall significantly during the period of observation (figure 1). It is of note that no change was seen until one and a half

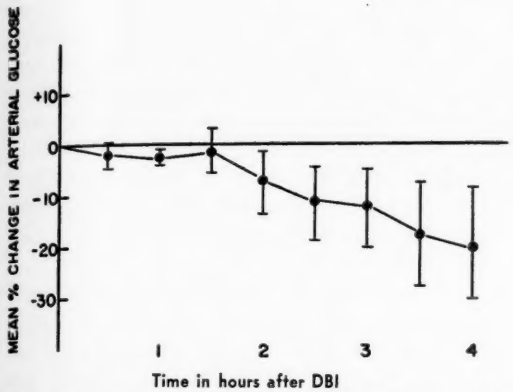

FIG. 1. Mean per cent change from control levels in arterial glucose concentration in five patients following 150 mg. DBI given by mouth.

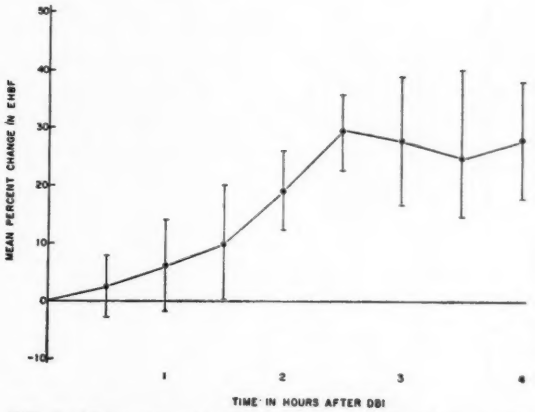

FIG. 2. Mean per cent change from control values in estimated hepatic blood flow (EHBF) in five patients following 150 mg. DBI given by mouth.

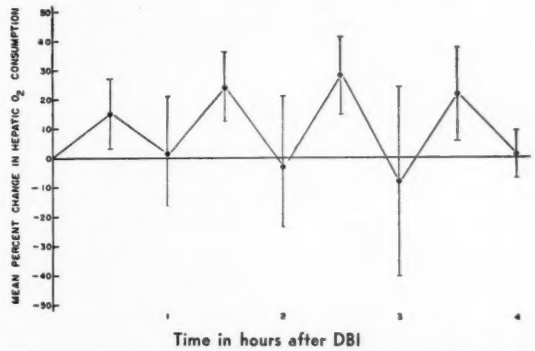

FIG. 3. Mean per cent change from control values in hepatic oxygen consumption in five patients following 150 mg. DBI given by mouth.

hours after the DBI was administered. Estimated hepatic blood flow (EHBF) is seen to increase significantly as much as 29 per cent above the control levels, an effect which also manifests its greatest change after one and a half hours (figure 2).

No consistent or significant change in hepatic oxygen consumption or glucose output was noted (figures 3 and 4). Because the sensitivity of the urea method was not sufficient to reflect consistently the small differences between arterial and hepatic vein blood, these figures have not been considered significant (figure 5).

Interpretation of the observations of hepatic glucose output (figure 4) is complicated by the marked variability of the glucose content of hepatic vein blood. These fluctuations have been demonstrated previously in the nondiabetic human by Bondy,[30] and in the dog by Anderson and his associates.[31] Inspection of the results of successive one-minute hepatic vein blood sugars (table 1) reveals that two patients (JB and JR) showed relatively stable levels over the period measured, while the remaining four patients showed remarkable instability, with fluctuations of 8 to 30 per cent between successive specimens. It is readily apparent that variations of this magnitude in one factor determining hepatic glucose output make interpretation of a significant change difficult unless marked or consistent effects are seen. In light of this, our results are interpreted as revealing no decrease in hepatic glucose output and no significant change following DBI administration.

TABLE 1

Hepatic vein blood sugar content of specimens drawn for the first forty-five seconds of six to eight successive one-minute periods, showing the rapidity and range of fluctuations of hepatic vein blood sugar content

	One-minute periods							
Pt.	1	2	3	4	5	6	7	8
CS	262.5	262	233.5	220.5	213.5	217.5	195	
JB	198	202.5	201	202	clotted	197.5		
JR	407.5	416	415	417.5	411	clotted		
AK	254.5	247.5	268.5	239.5	254.5	250		
EM	355.7	404.5	398	393	373	366.5	357.5	
CE	255.5	230	234.5	226	203.5	234.5	236.5	242

No significant change in arterial lactate or pyruvate levels was noted (figure 6). Hepatic vein lactate and pyruvate values were found to be consistently lower than arterial lactate and pyruvate and for this reason we have labeled the hepatic clearance of pyruvate and lactate as "hepatic lactate and pyruvate uptake." A highly suggestive increase in hepatic uptake of lactate and pyruvate was noted (figures 7 and 8). This increased hepatic clearance of pyruvate and lactate oc-

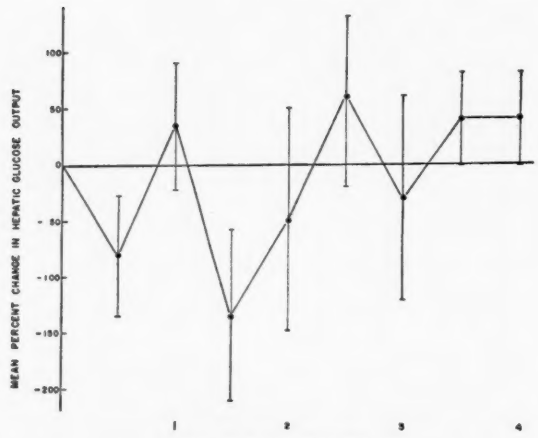

FIG. 4. Mean per cent change from control values in hepatic glucose output in five patients following 150 mg. DBI given by mouth.

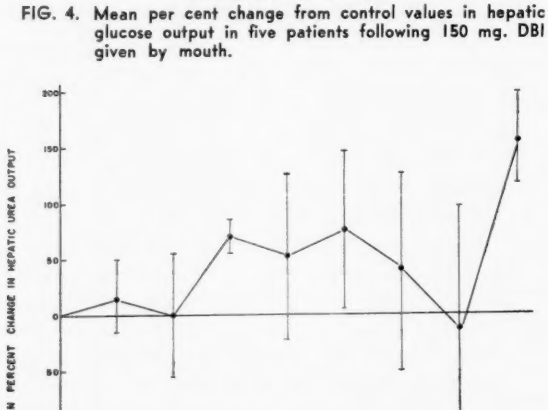

FIG. 5. Mean per cent change from control values in hepatic urea output in five patients following 150 mg. DBI given by mouth.

curred while arterial pyruvate and lactate levels remained relatively constant, and is apparently related to some extent to the effect of increased hepatic blood flow. The relationship of liver blood flow to hepatic lactate and pyruvate clearance has not been studied. The increase in hepatic uptake of lactate and pyruvate can only be explained by the increased production of these substrates in the peripheral tissues, apparently as a response to DBI. This is in keeping with previous observations[6,7,34,35] and serves as a reminder that the interpretation of changes in blood lactate and pyruvate levels may not be truly representative of pyruvate and lactate balance in the absence of measures of hepatic

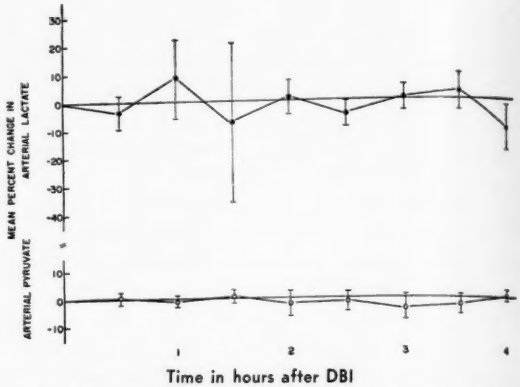

FIG. 6. Mean per cent change from control values in arterial lactate and pyruvate levels following 150 mg. DBI given by mouth.

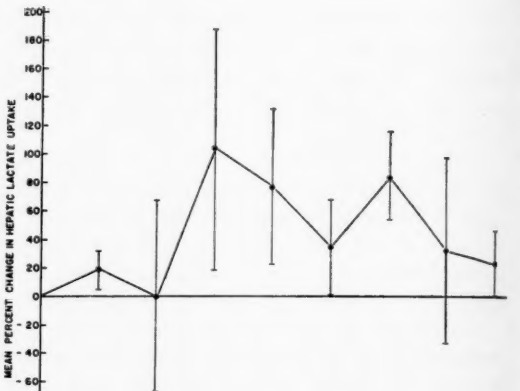

FIG. 7. Mean per cent change from control values in hepatic lactate uptake following 150 mg. DBI given by mouth.

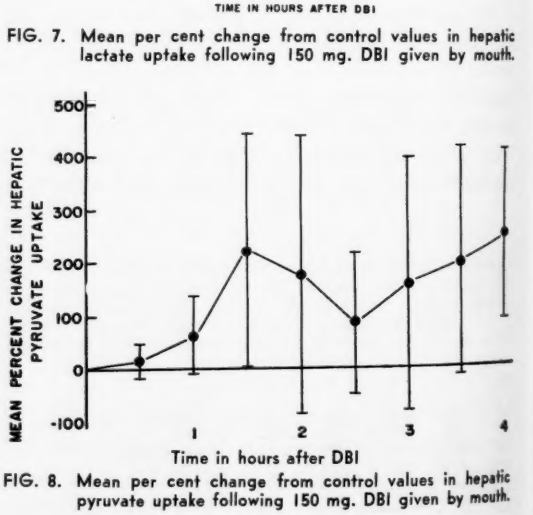

FIG. 8. Mean per cent change from control values in hepatic pyruvate uptake following 150 mg. DBI given by mouth.

removal of these substances. It is apparent that the capacity of the liver to clear lactate and pyruvate is such that highly significant changes can occur in the peripheral production of pyruvate and lactate in the absence of notable changes in arterial blood levels. From the present data, we are unable to estimate any change in the hepatic output of pyruvate and lactate, other than to state that any change in hepatic production of these substances is masked by the increase in uptake.

DISCUSSION

Some of the above results require further observation, particularly in the light of their variation from those of other investigators. These include the relationship of blood glucose level to hepatic glucose production and peripheral uptake of glucose, the metabolism of lactate and pyruvate, with reference to their origin and uptake by the liver, the observed increase in hepatic blood flow, and the relationship of arterial lactate and pyruvate as they reflect the status of oxidative metabolism.

The fall in blood glucose appears to be attributable largely to the activity of DBI as its onset parallels that of the other effects noted, and it was relatively consistent. Mosenthal and Lauber[26] noted a similar decrease in the blood sugar of untreated, fasting diabetics in 48 per cent of a mixed group of seventy diabetics studied in the fasting state for three months. However, their study included diabetes of all degrees of severity and an unstated number of these patients had received Protamine Zinc Insulin just twenty-four hours prior to the procedure.

The observation of decreasing arterial glucose levels in the presence of unchanged hepatic glucose output is illustrated in figure 9 where three subjects' findings are compared. These results, though not conclusive in this small number of subjects, suggest that DBI exerts its hypoglycemic effect by increasing the utilization of glucose peripheral to the splanchnic circulation rather than by decreasing hepatic glucose output or increasing hepatic glucose uptake. This interpretation does not exclude a hepatic mechanism of DBI activity, but it does suggest the action of a significant peripheral effect.

Of parallel significance are the changes noted in hepatic uptake of lactate and pyruvate. It should be pointed out that the failure to note an increase in arterial levels of pyruvate and lactate after DBI is not inconsistent with the findings by others of increased venous lactate and pyruvate.[14,15] The demonstrated increase in hepatic lactate clearance plus the dilution of

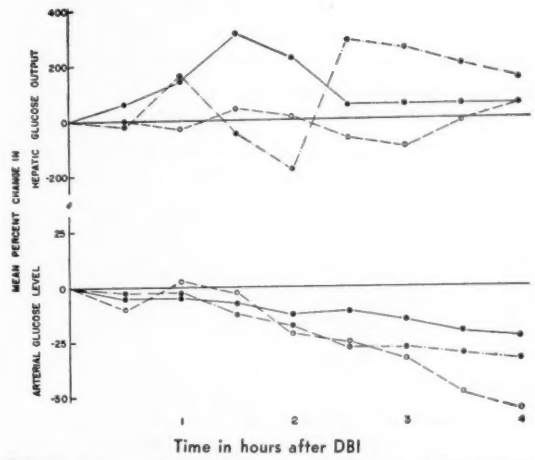

FIG. 9. A comparison of the per cent change from control values of hepatic glucose output and arterial glucose level in three patients following 150 mg. DBI by mouth, illustrating the occurrence of significant fall in blood glucose in the absence of a significant fall in hepatic glucose output.

peripheral venous blood in the arterial samples could account for the lack of change in arterial concentrations in the presence of increasing peripheral venous levels. Since venous lactate was not measured we can only assume that it was elevated. Regardless of this our results suggest that the production of lactate and pyruvate is increased in the peripheral tissues and that this increase is rapidly cleared by the liver.

The marked change in EHBF (figure 2) was unexpected and an adequate explanation is lacking. Some of the variables known to affect EHBF include disease of the liver, posture,[27] epinephrine and insulin release.[34,35] Our subjects may be considered stable during the experiment with regard to liver disease and posture. An hour was allowed for stabilization after all instrumentation was concluded, before measurements were begun, and all experiments included in the study were characterized by stability of the pulse and temperature of the patients throughout the procedure. The usual increase of hepatic glucose output seen with epinephrine administration did not occur and the consistent occurrence of the major increase in EHBF after one and a half hours does not suggest the rapid effect seen with epinephrine. For these reasons it is felt that the release of epinephrine does not account for the observed increase in EHBF, nor does hypoglycemia, a known stimulant of epinephrine release, as no hypoglycemic blood sugar levels were recorded. As these subjects were considered to be totally insulin-deficient, or nearly so, it

is felt that no significant insulin effect was recorded. An attempt is currently being made to verify further this effect of DBI on the EHBF by a different technic in a larger group of patients.

Huckabee[32-34] has recently demonstrated the existence of a constant relationship between the arterial level of lactate and that of pyruvate when concentrations of these substances are varied by manipulations which do not alter tissue oxygen supplies or oxidation-reduction potentials. He has used for the basis of his conclusions the mass action form (formula #2) of the formula for lactic dehydrogenase activity in the steady state (formula #1):

1. $(\text{Pyruvate}) \times (\text{DPN} \cdot \text{H}_2) = \text{K}\ (\text{Lactate}) \times (\text{DPN})$

2. $(\text{Lactate})_0 = (\text{Pyruvate})_0 \times \text{K} \dfrac{(\text{DPN} \cdot \text{H}_2)_0}{(\text{DPN})_0}$

Huckabee has shown from this relationship that in the relative tissue hypoxia induced by exercise or exposure to an atmosphere of low oxygen tension, lactate appears in excess of its expected ratio as estimated by a derivation of formula #2:

3. $\text{XL} = (\text{L}_n - \text{L}_o) - (\text{P}_n - \text{P}_o)\ (\text{L}_o/\text{P}_o)$

where XL is "excess lactate" produced in excess of that calculated for the steady state with respect to pyruvate concentration and oxygen tension, L_n and P_n are lactate and pyruvate concentrations at any time t_n and P_o and L_o are the levels observed in the basal state.

Huckabee has shown that the "excess lactate," or XL, produced is equal in energy relationship to the oxygen debt incurred by exercise. From these relationships it might follow that any significant activity of DBI in vivo in inhibiting oxidative glucose catabolism in the citric acid cycle as suggested by Wick and his associates[30] and Steiner and Williams[8] would significantly alter intracellular metabolism in such a manner as to affect the factor

$$\text{K}\frac{(\text{DPN} \cdot \text{H}_2)}{(\text{DPN})}$$

in a direction leading to the production of "excess lactate." A significant change in these relationships should be demonstrable by this method. The conditions set forth by Huckabee were present in these experiments in which frequent measures of arterial and venous oxygen content were available to eliminate the factor of induced tissue hypoxia. The calculations involved in formula #3 were performed with the results seen in table 2.

It is apparent from the values for XL which are either negative or small positive values, that no significant excess lactate is produced and it is therefore suggested that although the succinic dehydrogenase inhibition has been well demonstrated in vitro by DBI, this mechanism may not be of significance in acute DBI activity in vivo as determined within the limitations of the above experiment.

TABLE 2

Arterial blood lactate, pyruvate and oxygen saturation following administration of 150 mg. phenethylbiguanide by mouth, showing no calculated excess lactate formation (XL)

Pt.	Time *p* phenethylbiguanide min.	Lactate mg. per cent	Pyruvate mg. per cent	XL mM./L. plasma	O_2 sat. per cent
CS	0	8.22	1.23	0	94.3
	30	9.0	1.24	0.081	92.0
	60	8.0	1.25	—0.041	90.8
	90	7.3	1.21	—0.086	94.0
	120	6.85	1.25	—0.162	92.1
	150	6.8	1.29	—0.197	
	180	7.4	1.18	—0.049	
	210	7.35	1.22	—0.088	87.2
	240	7.50	1.28	—0.112	
VB	0	8.75	1.58	0	92.3
	30	9.7	1.47	0.038	97.1
	60	9.25	1.5	0.105	96.0
	90	9.15	1.72	—0.042	97.8
	120	9.65	1.69	0.032	86.3
	150	9.35	1.58	0.067	87.9
	180	9.7	1.65	0.062	89.8
	210	9.15	1.62	0.019	82.5
	240	9.25	1.61	0.037	86.0
AK	0	2.71	1.79	0	96.5
	30	2.30	1.76	—0.052	92.8
	60	4.46	1.83	0.188	
	90	4.92	1.75	0.252	88.9
	120	3.21	1.54	0.089	
	150	2.41	1.52	0.011	91.5
	180	2.47	1.41	0.026	
	210	2.35	1.64	—0.016	91.5
	240	1.87	1.66	—0.072	
EM	0	9.48	0.87	0	97.1
	30	8.05	0.83	—0.106	
	60	8.25	0.84	—0.091	94.7
	90	8.0	0.83	—0.112	
	120	8.95	0.76	0.078	96.6
	150	8.35	0.83	0.038	
	180	9.55	0.83	0.044	93.1
	210	7.15	0.79	—0.245	
	240	7.15	0.88	—0.256	93.7
JR	0	9.55	2.38	0	95.1
	30	8.7	2.38	—1.155	97.7
	60	8.25	2.36	—1.196	99.2
	90	7.7	2.44	—2.080	99.1
	120	9.5	2.52	—1.129	
	150	9.45	2.51	—1.129	98.2
	180	10.5	2.55	—1.219	
	210	10.45	2.52	—1.223	94.8
	240	10.4	2.51	—1.269	

SUMMARY

The acute effects of an oral dose of 150 mg. phenformin were studied in five patients over a four-hour period by the technic of right hepatic vein catheterization. A sig-

nificant fall in arterial glucose levels was seen while no consistent change was noted in hepatic glucose output. This suggests that increased peripheral utilization of glucose is an acute effect of phenformin.

Estimated hepatic blood flow was seen to increase significantly, as did hepatic uptake of pyruvate and lactate, while arterial levels of pyruvate and lactate remained stable throughout the period of study. These findings support previous observations of increased production of lactate and pyruvate peripheral to the splanchnic circulation and add the observation of increased hepatic removal of these substances.

No consistent or significant effect of phenformin was recorded on hepatic oxygen consumption or urea production. Evidence is presented of the rapid and gross variations which may occur in hepatic vein blood sugar.

Finally, it was demonstrated that the ratio of arterial lactate to arterial pyruvate levels remained unchanged, suggesting that no significant intracellular anoxia resulted from the acute administration of phenformin.

SUMMARIO IN INTERLINGUA

Certe Effectos de Biguanida Phenethylic in le Metabolismo Hepatic de Humanos, Mesurate per Catheterismo de Vena Hepatic

Le effectos acute de un dose oral de 150 mg de biguanida phenethylic esseva studiate in cinque patientes durante un periodo de quatro horas per medio del technica de catheterismo hepatic dextero-venose. Un grado significative de reduction del nivellos arterial de glucosa esseva constatate, sed nulle altere effecto uniforme esseva apparente in le rendimento de glucosa hepatic. Isto pare indicar que le utilisation peripheric de glucosa es un effecto acute de biguanida phenethylic.

Le estimation del fluxo de sanguine hepatic monstrava valores significativemente augmentate. Etiam le acceptation hepatic de pyruvato e de lactato esseva augmentate, durante que le nivellos arterial de pyruvato e de lactato remaneva stabile durante le integre periodo del studio. Iste constatationes supporta previe observationes de un augmento del production de lactato e pyruvato in sitos peripheric con respecto al circulation splanchnic. A iste observationes es addite le constatation de un augmento del elimination hepatic del mentionate substantias.

Nulle uniforme o significative effecto de biguanida phenethylic esseva notate in le consumption hepatic de oxygeno o in le production de urea per le hepate. Es presentate datos indicante un rapide variabilitate possibile in le sucro del sanguine venose in le hepate.

Finalmente, esseva demonstrate que le proportion inter le nivellos arterial de lactato e pyruvato remaneva inalterate. Isto pare indicar que nulle significative anoxia intracellular resultava ab le acute administration de biguanida phenethylic.

ACKNOWLEDGMENT

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Additional evidence for the superiority of complex carbohydrates over the simpler sugars in "sparing protein" comes from the work of the Wisconsin group. A. E. Harper and M. C. Katayama (*J. Nutrition* 49:261, 1953) showed that on a 9 per cent casein ration containing 0.5 per cent methionine, weanling rats gained 25 gm. per week (average for the four-week period) when the carbohydrate was starch (other ingredients in per cent: corn oil 5, salts 4, and adequate vitamins). The growth rate was 10 to 13 gm. per week when sucrose replaced the starch. On 18 per cent casein diets, the rats gained 35 and 38 gm. on the sucrose and starch rations, respectively.

When the low casein-sucrose ration was supplemented with liver extract, extra vitamins, an antibiotic-vitamin B_{12} mixture or the unessential amino acids, there was no increase in growth rate. The addition of essential amino acids, which are limiting in a 9 per cent casein ration (0.4 per cent lysine, 0.15 per cent valine, 0.2 per cent threonine, 0.1 per cent tryptophan and 0.2 per cent histidine), increased the growth rate to that of the starch group (25 gm. per week). A similar improvement in the growth of the rats fed the sucrose ration was seen when the level of casein was raised to 12 per cent. When dextrin (produced by heating moist corn starch in an autoclave at 15 lbs. pressure for three hours) was used in place of sucrose, growth on the 9 per cent casein ration was the same as that with corn starch.

The hypotheses put forth to explain the superiority of complex carbohydrates over the simpler sugars were: (1) The less soluble carbohydrates favor the synthesis of the essential amino acids by the intestinal microflora; (2) improved digestion and absorption of protein occurred as the result of the slower passage of the complex carbohydrates through the gastrointestinal tract; (3) there was a reduced destruction of the amino acids in the animal as a result of the prolonged absorption of glucose from the insoluble carbohydrates (A. E. Harper and C. A. Elvehjem, *J. Agricultural and Food Chemistry* 5:754, 1957).

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Special Article

Basic Clinical Metabolic Studies with Phenformin

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Leonard L. Madison, M.D.
Dallas

James W. Craig, M.D.
Cleveland

Thomas G. Skillman, M.D.
Columbus

Stefan S. Fajans, M.D.
Ann Arbor

Robert E. Tranquada, M.D.
Los Angeles

MODERATOR DANOWSKI, (*Pittsburgh*): I would like to suggest to the panel that we first consider the effects of DBI, if any, on the degradation of insulin, whether it be endogenous or exogenous. Could we disregard this as a possible mechanism of DBI action?

THOMAS G. SKILLMAN, M.D., (*Columbus*): We believe that altered degradation could not account for the hypoglycemic effect of DBI, but decreases in the rate of insulin degradation under the influence of DBI have been reported.

MODERATOR DANOWSKI: On the other hand there are data which indicate that DBI does not alter degradation of insulin in the in vitro systems. We are left, therefore, with some uncertainty. Let us ask Dr. Bergen to comment further on his studies of adrenocortical function in patients receiving DBI.

STANLEY S. BERGEN, JR., M.D., (*New York City*): I think our studies indicate that DBI exerts no gross effects upon adrenocortical function as reflected in the urinary levels of 17-hydroxycorticosteroids and 17-ketosteroids. This type of evidence excludes, of course, the possibility that the observed hypoglycemic effects of DBI were attributable to hypoadrenocorticism. If anything, there was an increased response to DBI, particularly in one patient, following the administration of ACTH.

MODERATOR DANOWSKI: It is conceivable, is it not, that there might have been changes in the production of endogenous ACTH in your patients?

DR. BERGEN: There certainly might, but all our base-line levels were normal. If there was an actual block of pituitary-adrenocortical activity, I would expect the urinary steroids to be at least low normal.

MODERATOR DANOWSKI: I would like to turn your attention now to the DBI-thyroid data. These indicated, you will recall, that DBI did not alter the serum protein bound iodine nor the net trapping of I^{131}. The trend toward decreased trapping which was evident did not prove to be statistically significant. I would like to ask Dr. Skillman, however, whether these findings entirely exclude changes in the levels of circulating hormones and the trapping of iodine?

DR. SKILLMAN: We have considered these possibilities. At times diabetics have a defect in their alpha globulin, i.e., in the thyroxine binding globulin. We too wonder if protein-bound iodine values are always directly reflective of thyroid physiology and thyroid hormone metabolism. Also, insofar as the I^{131} trapping is concerned, our studies do not exclude the possibility of transient early inhibition of iodine uptake which is compensated for later on.

MODERATOR DANOWSKI: Does anyone have any data on epinephrine tolerances with and without DBI? These might prove enlightening because of the increase in lactic acid induced by this hormone.

DR. SKILLMAN: We studied three patients before and after DBI with standard epinephrine tolerances, measuring blood sugar at twenty-minute intervals for an hour. The rise was the same with and without DBI. We did not measure lactate.

MODERATOR DANOWSKI: I would now like to ask Dr. Craig and Dr. Fajans to comment further on their lactate and pyruvate data. You will recall that both of them expressed the view that the rises in these products of glycolysis represented increased production or diminished disposal. Theoretically, there are three other

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possible explanations for the observed increases in the levels of lactate and pyruvate: Both increased production and diminished disposal could occur; production and disposal could both be decreased with the latter predominant; and finally, both increased production and increased disposal could be present with the former predominant. Thus in one of Dr. Craig's studies there was suggestion that both the production and the disposal of lactic acid were increased by DBI.

JAMES W. CRAIG, M.D., (*Cleveland*): We have a problem in how to express the rate of disappearance of lactate. One can speak of the fractional rate of disappearance in terms of increase above the fasting level, or one can speak of the rate of disappearance in terms of the total elevation of lactate.

If one does it in terms of the increase above the fasting level, the fractional rates of disappearance after sodium lactate was administered were similar with and without DBI, although the total rise was somewhat greater under the influence of DBI. If one takes into account the higher absolute level, then this is a relatively slower rate of disappearance of lactate.

STEFAN S. FAJANS, M.D., (*Ann Arbor*): As far as the question of increased production versus decreased removal, or any number of combinations of these two factors, one can only speak of the algebraic sum or the net effect in the studies that we have done. We found that there was a decreased disappearance of pyruvate and of lactate from blood. I think when we say decreased disappearance, this takes into consideration the possibilities you have mentioned.

MODERATOR DANOWSKI: Dr. Craig, do you think that lactate production is increased?

DR. CRAIG: I do not think from our data that we are able to make a decision on that particular point. I think there is an alteration in equilibrium, and it is increased in that sense.

ROBERT E. TRANQUADA, M.D., (*Los Angeles*): I would like to add that our data suggest that there is both increased production of lactate peripheral to the liver and increased uptake in the liver.

MODERATOR DANOWSKI: Where is the lactate being produced?

DR. TRANQUADA: We did not measure venous levels, but we presume the lactate is produced by the peripheral tissues and that increased uptake by the liver prevents arterial levels from rising.

DR. CRAIG: With repeated administration of the drug in larger doses, we do find elevations of lactate levels in arterial blood.

MODERATOR DANOWSKI: Let us now turn to the subject of gluconeogenesis. Dr. Madison, how about the extrahepatic production of glucose?

LEONARD L. MADISON, M.D., (*Dallas*): The only significant source of extrahepatic glucose is, as far as I know, the kidney and gluconeogenesis in that organ only occurs under unusual circumstances.

MODERATOR DANOWSKI: Here is one question that I am sure one of you can answer: Does DBI potentiate exogenous insulin?

DR. FAJANS: If this were true, one should find a change in insulin sensitivity and in glucose tolerance. We could not find any such changes, and we therefore feel that DBI does not potentiate the activity of insulin.

DR. MADISON: We have some evidence of a preliminary nature which shows that when we infuse exogenous insulin at a very slow rate, we observe no change in arteriovenous glucose difference. When insulin is given at the same rate with DBI there is apparently a widening of the glucose arteriovenous difference that may indicate potentiation of insulin action.

DR. FAJANS: I do not believe that just because insulin was necessary for the effect of DBI to be manifested, that insulin activity was potentiated. One has to differentiate between the permissive action of a hormone and actual participation of a hormone.

DR. BERGEN: We had a forty-six-year-old patient who had been subjected to a pancreatectomy one year earlier. He was on a high caloric intake, and forty units of long-acting and twenty units of short-acting insulin a day. On this regimen he was spilling 30 to 40 gm. of glucose in twenty-four hours. When we began to add DBI, we got to a point where we were administering over 300 mg. of DBI and had reduced the insulin dosage to ten units. The twenty-four-hour urine then contained 9.7 gm. of glucose. The patient developed anorexia on this dosage and his caloric intake could no longer be maintained.

This indicates that DBI was able to replace a certain amount of insulin, and decrease the carbohydrate wastage in urine. Whether this is potentiation or just straightforward replacement of a certain number of units of insulin, we are unable to say.

MODERATOR DANOWSKI: I would not believe this observation answers the fundamental question. The findings are compatible with an action which is similar to that of insulin or compatible with potentiation. Therefore, it is still moot.

DR. TRANQUADA: We had a similar experience with a twenty-year-old male who required seventy-five units of NPH and twenty-five units of Regular Insulin. On this regimen the spillage of glucose was 10 to 30

gm. per day. We began treating him with DBI and within a two-week period had increased the DBI to 300 mg. a day and decreased his insulin to 25 U. per day. At this stage his urine was sugar-free for four consecutive days, and the blood sugar level was around 80 mg. per 100 ml.

On the day we stopped his insulin he went into severe acidosis with elevation of the blood sugar level to over 300 mg. per 100 ml. He had to be given insulin to get him out of that acidotic episode.

MODERATOR DANOWSKI: Someone asked about the concentration of DBI in the gastric juices of patients.

MAX MILLER, M.D., (*Cleveland*): When labeled DBI was administered in large amounts, the label appeared in the gastric juice. These were studies in animals.

MODERATOR DANOWSKI: What was the label?

DR. MILLER: Carbon 14.

MODERATOR DANOWSKI: Unfortunately, labels have a life of their own. If you label with iodide, the localization of the label does not necessarily indicate what happened to the compound. It indicates what happens to the iodide.

Is there any localization of DBI in the rest of the intestinal tract? Does anyone know?

ARNE WICK, PH.D., (*San Diego*): I would say that most of it is in the gastric juice. While I am on my feet, may I make a statement on this question of lactate. I would like to put in a plea for caution in the interpretation of lactate studies. I think Dr. Miller will bear me out. Lactate is one of the most difficult things to study, although one can measure it easily in the blood. The reason it is difficult to study is that it is immediately converted to CO_2 after injection.

I would also like to speak on the point of tissue sources of glucose. No one has demonstrated that glucose will diffuse out of muscle cells. As a matter of fact, no one has demonstrated free sugar inside the cell under normal conditions. Now, if a diaphragm is exposed to sugar at 2,000 mg. per cent, which I think is worthless, you can demonstrate free sugar. You can also produce free sugar in the kidney only under hyperglycemic conditions. We did that several years ago.

MODERATOR DANOWSKI: The point that you make about lactate is a good one, and yet I was impressed by the narrow range of responses that Dr. Craig and Dr. Fajans observed in their patients, suggesting that perhaps even though the limitation you cite is present, that it is of equal degree with and without DBI.

Here is another question from a member of the audience: Does DBI increase ketone formation in the total diabetic not given insulin?

DR. TRANQUADA: The patient I was referring to spilled increasing amounts of ketones in the urine only when we discontinued insulin not because we gave DBI.

DR. SKILLMAN: Our studies in rabbits suggest that ketosis is not produced by DBI.

MODERATOR DANOWSKI: Is the panel now ready to express itself on the mechanism or mechanisms of action of DBI? Is the hypoglycemic effect related to a decreased output of glucose by the liver?

DR. TRANQUADA: No, I don't think we can draw that conclusion from what we have seen thus far and I think that is as far as we can go.

DR. MADISON: I can only say again what I said before, it doesn't seem to be working on the periphery in the muscle. It may work on fat which we didn't study and it may work on the liver. From Dr. Tranquada's data, it doesn't appear to work on the liver.

DR. MADISON: I would like to speak further on the subject of lactate. When man is in the postabsorptive state, most of the glucose that is utilized by the muscles is utilized through anaerobic metabolism. If I am correct, 60 to 70 per cent of the glucose thus utilized ends in lactate production. There is nothing inherently wrong with that. Such a process can produce energy if the lactate is then metabolized. I believe this is exactly what does happen. Since the lactate is not lost in the urine, it must be metabolized somewhere. There are two ways in which glucose can be used by the diaphragm. Under the influence of insulin, it may go to glycogen. DBI does not appear to stimulate this pathway. On the other hand, when glucose is present at higher levels, this amount utilized is increased and lactic acid is formed. It may be that DBI facilitates this pathway.

MODERATOR DANOWSKI: Perhaps the reason why the importance of anaerobic glycolysis tends to be minimized is that from the point of view of energy production the Krebs cycle is much more effective. Yet as Dr. Madison points out, anaerobic glycolysis is an effective way of producing compounds which can then be utilized in what may well ultimately be energy-producing reactions.

DR. TRANQUADA: But if this is the fact, one would have to utilize more glucose through the glycolytic cycle and the glucose has to come from somewhere, probably the liver, and the two theories, decrease of glucose output and increase in anaerobic glycolysis, are not compatible.

MODERATOR DANOWSKI: You are saying that anaerobic glycolysis is a relatively inefficient mechanism compared to the Krebs cycle in terms of energy production and therefore would require larger amounts

of glucose. What then would be the source of this glucose?

DR. MILLER: It could be glucose that would otherwise be lost in urine.

DR. FAJANS: In one of our patients on DBI the urinary glucose was reduced from 160 gm. down to 30 gm.

MODERATOR DANOWSKI: Logically, we should now proceed to a discussion of possible effects of DBI on the Krebs cycle.

DR. CRAIG: We have studied citric acid, measuring the blood concentrations and the twenty-four-hour urinary excretion. We have not been able to demonstrate increases in citric acid excretion or citric acid levels, although I think there are animal studies in which increases have been observed.

MODERATOR DANOWSKI: Dr. Ungar, you have done some work in animals on this point.

GEORGES UNGAR, M.D., Sc.D., (*New York City*): DBI does have a striking effect on the Krebs cycle when given in large dosages to animals. The detailed changes were discussed, as you know, this morning.

MODERATOR DANOWSKI: Are any of you troubled by the fact that an increase in anaerobic glycolysis might be undesirable?

DR. TRANQUADA: It might be discussed on slightly different terms. It is conceivable that the operations of the Krebs cycle are reduced in the muscle and not in the liver. I don't know whether this is a valid consideration, but it is a possibility that has to be considered.

QUESTION FROM THE AUDIENCE: Dr. Fajans cited a patient in whom the urinary glucose fell and the blood sugar fell following DBI but the ketone levels rose. Isn't that an indication that the DBI was stimulating another pathway of metabolism leading to ketone production?

DR. FAJANS: I don't think we can say that there was any evidence of increased ketone production produced by DBI. I agree with Dr. Skillman's previous statement that the ketosis resulted from withdrawal of insulin.

A MEMBER OF THE AUDIENCE: I'd like to comment on this point. If the diet is low in calories and carbohydrate, the patient will show ketonuria. If the diet is increased, the ketonuria disappears. This is considered to be a starvation effect and not a product of DBI itself.

QUESTION FROM THE AUDIENCE: Doesn't it suggest that a patient on DBI needs more carbohydrate than a patient on insulin only?

MODERATOR DANOWSKI: This takes us back to Dr. Tranquada's idea and is a very large question. I think that diabetics are going to get more carbohydrate in the future, because they are getting too much fat at present; but there is no evidence that DBI increases the requirement for carbohydrate.

QUESTION FROM THE AUDIENCE: The usual ketone level is a little over 1 mg. per cent in the blood of the nondiabetic. In diabetic patients regarded as being in good control on insulin, the ketone level is two or three times higher, i.e., between 2 and 3 mg. per cent.

In patients treated with DBI, the blood ketone level rises three- or fourfold above the basal values.

Do we have any clues why DBI does not work in nondiabetic subjects with endogenous insulin?

MODERATOR DANOWSKI: An excellent question. I'd say it is because you cannot improve upon success.

DR. SKILLMAN: I do not have an answer, but I can add another question. We have not seen hypoglycemia develop with DBI as it does with the sulfonylureas and one might wonder about a built-in glucostat whereby DBI might be more effective at higher concentrations of blood sugar and less effective at lower concentrations.

MODERATOR DANOWSKI: How large have the doses of DBI been in the studies in nondiabetic humans?

DR. SKILLMAN: We have given up to 400 mg. and obtained slight decreases in blood sugar.

DR. FAJANS: We gave up to 400 mg., too, and as you saw observed a slight decrease. This is a minimal change which is not statistically significant. Dr. Madison made a very significant observation, if I understood him correctly; diabetic patients with fasting blood sugar in the normal range did have a hypoglycemic response and the normals did not. Did I understand you correctly?

DR. MADISON: Yes, we encountered such a patient. We didn't see a diabetic or nondiabetic on DBI go below 70 mg. per cent.

DR. MILLER: I am led to believe that no one has seen hypoglycemia.

MODERATOR DANOWSKI: Dr. Ungar, I hope that you will comment upon hypoglycemia following large dosages of DBI given to animals.

DR. UNGAR: I believe it is purely a quantitative matter. I have some evidence from studies in monkeys. In normal monkeys you do not get any hypoglycemia with DBI dosages below 5 mg. per kg. of body weight. At dosages above 8 mg. per kg. you have a drop of 8 per cent in the level of blood sugar.

On the other hand, we have seen one diabetic monkey whose diabetes was perfectly controlled by 1 to 2 mg. of DBI per kg.

I believe that if humans were given DBI at a level corresponding to 5 mg. per kg., more hypoglycemia would be observed.

DR. FAJANS: The dosage used in our normal subjects extended up to 5.7 mg. per kg. but a hypoglycemic effect was not observed.

DR. MILLER: Did the monkey respond to DBI at 5 mg. per kilo?

DR. UNGAR: Yes, and he was controlled.

DR. FAJANS: A person with diabetes may, and I would like to underline with a red pencil the word "may," have an abnormality in the removal of pyruvate. This has been referred to in several papers as a possibility. If this should be so, and if DBI interferes with the removal of pyruvate, we would anticipate a greater effect of DBI in diabetic subjects than in normal ones.

DR. MADISON: If you can't get rid of pyruvate and if you block it more, it would mean that the blood sugar would rise and not fall.

DR. CRAIG: May I ask Dr. Fajans a question in regard to this postulated theory in pyruvate. Have you tested the effect of DBI on a patient with Cushing's syndrome, since these may be more marked than in an ordinary diabetic?

DR. FAJANS: No, we have not.

MODERATOR DANOWSKI: I am afraid that in these at times lengthy discussions we have not settled many of the problems posed by ourselves and by the audience. It might be well to summarize the consensus on some of these.

Though the evidence is not entirely consistent, the hypoglycemic action of DBI does not appear to result from a decrease in the degradation of endogenous or exogenous insulin.

DBI does not potentiate the action of exogenous insulin judging from insulin tolerance tests and from glucose tolerance tests in subjects with some presumed residual production of insulin. Similarly there appear to be no striking or persistent effects of DBI upon adrenocortical or thyroid function nor upon epinephrine-induced hyperglycemia.

The accumulation of lactate and pyruvate under the influence of DBI is definite. Though alternative explanations might be offered, this accumulation could be the result of both increased production and blockade of disposal of glycolytic products via the Krebs cycle.

Evidence is available that, if some insulin is available so that anaerobic glycolysis is occurring, DBI accelerates the operation of the hexosemonophosphate shunt. Though some concern has been expressed concerning the possible harmful effect of acceleration of anaerobic glycolysis, none has been observed to date. It would appear that the drug accelerates the metabolic disposal of glucose and in the diabetic but not the nondiabetic lowers blood sugar levels. Though much is yet to be learned about its mechanism of action, it is already clear that DBI is a prototype of a new group of agents which will add much to our understanding of intermediary metabolism and ultimately lead to more adequate regulation of diabetes mellitus.

Small differences in caloric expenditure which may represent a great accumulation of body fat over a period of time are difficult to demonstrate with statistical assurance. It must always be remembered that measurements upon a relatively small group or with few tests cannot be concluded to be the same simply because they are not statistically different. It is unfortunate that the measurements in this study could not have been repeated, to define the apparent differences between groups and meals with more assurance.

There was shown to be a significant positive correlation between the fasting blood glucose and the degree of deviation from normal weight patients. The correlation, however, was not high ($r = .58$). There was also some indication of a more rapid rise in blood glucose and perhaps a greater decrease, to below fasting levels, in the overweight group after a high carbohydrate meal than in the other groups. Fasting blood pyruvic acid concentrations also tended to be high in the overweight group.

The measurements of various serum lipids failed to reveal significant differences in the . . . groups. These included total serum lipids and cholesterol, various lipoprotein fractions, and chylomicron counts. Large individual differences within each of the groups, as would be expected from the literature (J. W. Gofman et al., *Circulation* 14:691, 1956) were found for these serum constituents. The fasting chylomicron counts tended to be high in the obese group.

However, as long as differences can be shown, new studies suggest themselves. There can be little doubt that the obesity problem is largely one which relates to the disposition of energy. Many of the observations which have been recorded require much more extensive documentation. From *Nutrition Reviews*, Vol. 15, No. 6, p. 165, June 1957.

Phenformin in the Management of Diabetes Mellitus

General Clinical Concepts

Martin G. Goldner, M.D., Brooklyn, New York, Moderator

Robert S. Baldwin, M.D.
Marshfield, Wisconsin

Harold L. Dobson, M.D.
Houston

Leo P. Krall, M.D.
Boston

Thomas H. Lambert, M.D.
La Jolla, California

Emery C. Miller, M.D.
Winston-Salem

Julius Pomeranze, M.D.
New York City

Charles Weller, M.D., Valhalla, New York

This first of the two clinical panel discussions dealt with general aspects of the management of diabetes mellitus with phenformin (DBI). The seven panelists reported experiences with a total of about 1,000 patients, with half of this number observed for more than six months. The series of Krall and of Pomeranze were as large as 300 and more patients, many of them under treatment for more than two years. The series of Dobson, Lambert and Weller comprised 70 to 100 patients; Baldwin and Miller reported on twenty-seven and thirty-one patients respectively. The patient material included both maturity-onset and growth-onset diabetes, "brittle" diabetes and the various stages of severity of the disease as judged by insulin requirement on the one hand, and the presence or absence of complications on the other. An appraisal of therapeutic results is usually difficult in the absence of uniform criteria for selection of patients or for control, particularly so in diabetes where not only sound definitions as to what constitutes good control are badly lacking, but where also spontaneous fluctuations in the nature of the disease must be considered critically. While most of the panelists had selected for their study patients who had been treated previously with moderate doses of insulin or with other oral agents, some had included in their series patients who responded to dietary restrictions alone. Nevertheless, a rather impressive concurrence of observations became evident from the presentations of the various panelists, regardless of whether the strongest criteria of normoglycemia and aglycosuria were applied, or the lenient ones of maintenance of weight, symptom relief and establishment of well-being, and whether juvenile, brittle or growth-onset diabetes was discussed.

1. DBI is an effective hypoglycemic agent with an activity similar to that of the hypoglycemic arylsulfonylureas. Because of its short action curve it must be administered three times, or even four times daily.

2. Its clinical usefulness is limited by the development of nausea and vomiting and other signs of gastrointestinal incompatibility which occur with increasing frequency in doses higher than 150-200 gm. per day. Its daily dosage should therefore not exceed a maximum of 200 mg.

3. No toxic reactions in the hematopoietic or connective tissue systems, the liver or the kidney, or any other system tested, were observed, nor was there any evidence of severe hypoglycemic reaction.

4. Ketonuria develops not uncommonly in patients treated with DBI; this is interpreted as a sign of starvation ketosis and can easily be overcome by liberalizing the diet. It must be differentiated clearly from the ketonuria attending impending acidosis or aggravation of the diabetes.

5. There are two areas of clinical usefulness of DBI: (a) *As sole medication,* it may be used in those diabetic patients with maturity-onset of the disease who cannot be controlled on diet alone, and who have a moderate insulin requirement, or who respond favorably to other oral hypoglycemic agents. (b) *In conjunction with insulin or other hypoglycemic agents,* it may be used in the juvenile or growth-onset diabetes and in brittle diabetes, where it may eliminate the frequently wide blood sugar fluctuations, stabilize the glycemia

Presented at the Symposium on "A New Oral Hypoglycemic Agent, Phenformin (DBI)" in Houston on Feb. 6, 1959.

and permit a decrease in the insulin dose.

Dr. Pomeranze's experience with diabetic patients older than forty-five years revealed no instance of drug sensitivity or toxicity. The chief cause of failure, however, appeared to be the development of gastrointestinal symptoms. These appear to be central in origin, do not respond to drug therapy, but are fortunately easily and precisely recognizable, and thus may act as a safety device, preventing overdosage. Pomeranze considers 200 mg./day as the upper limit of dosage.

All panelists agreed that with better knowledge of the limitation of the drug and better selection of the potentially responsive patients, not more than 10 to 15 per cent primary failures will be incurred and that if doses not higher than 200 mg./day are employed, incompatibility will necessitate discontinuation of the drug in not more than 20 to 35 per cent of responsive patients. Previously reported higher rates of failure or incompatibility were mainly due to inexperience in dosage and in selection of patients during the early trial periods.

Divided medication usually three times daily of 25-50 mg. each was recommended generally. This is necessary because of the relatively short action curve of DBI, which is about six hours. In some instances even four daily doses were used with the fourth dose given at bedtime (Krall).

In a study of seventy-one unselected diabetic patients who had been on restricted diets, Lambert found evidence of better control in the adult labile or brittle diabetic and in the juvenile patient receiving combined DBI and insulin therapy. Fifty-four per cent of these patients have been adequately controlled on DBI alone or in combination with insulin. Demonstrable blood sugar reductions occurred in 74 per cent of his patients receiving the compound. But here, too, the gastrointestinal side effects of anorexia, nausea, vomiting, and diarrhea were serious in as many as 40 per cent of patients. They subsided with lowered dosage. A suggestion of antithyroid activity by DBI was noted in two of six patients in whom the uptake of I^{131} was depressed. Dr. Lambert suggested that the drug may inhibit a reaction in iodine metabolism catalyzed by peroxidase.

Combined insulin-DBI therapy was uniformly regarded as a distinct progress in the management of the juvenile and the brittle diabetes and as a unique advantage of DBI (Krall). Combined treatment with other oral agents was found to be advantageous as well. Such combined treatment should, however, be reserved for those situations in which careful trials with one agent alone (insulin or the oral drugs) do not permit satisfactory control. Where one agent alone is effective, combined treatment may not serve any useful purpose, even if it permits a decrease in the dose of one of the drugs.

In discussing his series of ninety-three patients drawn from a charity outpatient clinic with preponderance of poorly controlled cases with many complicating diseases, Dobson found his experience to fall pretty much in line with that generally reported. None of his patients receiving DBI alone experienced any episode of severe hypoglycemia, and side effects severe enough to warrant cessation of therapy occurred in only 12 per cent of patients. He, too, found DBI particularly useful to improve the control of the brittle juvenile and poorly controlled patients, usually in conjunction with insulin.

Although some hypoglycemic effect of DBI is noted in the alloxan diabetic animal and in "surgical diabetes" of man, the clinical use of this compound in diabetes after total pancreatectomy is considered contraindicated as are the arylsulfonylurea compounds. DBI was found to be adequate, however, for the continued control of responsive patients during minor complicating diseases and during surgical complications (Dobson, Pomeranze). It was found effective also in instances of tolbutamide-escape, and observations were reported where tolbutamide sensitivity was restored after a period of treatment with DBI.

No experiences are available as yet concerning the effect of DBI on the degenerative diabetic complications, neuropathy, nephropathy, and angiopathy. While the drug has been used widely in these cases and no unusual progression has occurred, the periods of observation are still too short to expect evidences of regression or of prevention. Thus, Weller, studying patients for about eighteen months, has detected no apparent influence on the vascular complications of diabetes. But he too observed that only long-term studies will serve to evaluate this problem as well as that of any serious prolonged side effects that may as yet be undetected. Of seventy-one patients studied by his group, 65 per cent were described as totally responsive, while 35 per cent were deemed nonresponsive to DBI therapy.

Finally, Miller as well as Baldwin, in reporting their experiences joined the remainder of the panel in concluding that DBI when properly employed is a useful addition to the armamentarium for treatment of diabetes. But they too emphasized that many questions, particularly those related to the mechanism of action of the drug and of long-term toxicity are still unanswered, and that only the future will teach us whether the general clinical concepts presented at this panel will stand the test of time.

Phenformin in the Management of Diabetes Mellitus

Special Problems

Samuel B. Beaser, M.D., Boston, Moderator

Henry Dolger, M.D.
New York City
Barnett Greenhouse, M.D.
New Haven
Elmer Kleefield, M.D.
New York City
William Pearlman, M.D.
Wilkes-Barre
Frank Scott Perkin, M.D.
Detroit
Maurice Protas, M.D.
Washington, D.C.
Robert S. Radding, M.D.
Houston
Priscilla White, M.D.
Boston

Herewith are summarized eight discussions on the evaluation of DBI beyond its use as the sole therapeutic agent in the treatment of diabetes mellitus. These were presented by the panel on special clinical applications of DBI at the symposium on phenformin.

It is apparent from previous work that DBI lowers the blood sugar and controls the glycosuria in some cases of diabetes, but does not fully control some of the other interrelated metabolic factors.[1-6] Therefore, in the clinical application of the drug, DBI requires the presence of insulin, whether of endogenous origin, as in the maturity-onset type of diabetes, or exogenous origin, particularly in the juvenile diabetic. Thus, it is used in three basic ways:

1. Alone, to supplement the endogenous and avoid the need for exogenous insulin. In some patients reported in the symposium, the drug thus employed proved successful following failure of therapy with the arysulfonylureas. The use of DBI alone is especially effective in maturity-onset patients who have significant amounts of residual endogenous insulin.

2. In the same group and for the same purpose, as a supplement to the sulfonylurea drugs (Dolger, Pearlman).[2]

3. In the juvenile type of diabetes with a paucity of endogenous insulin, to supplement exogenous insulin, thus often reducing the insulin dosage. In the latter group, a further important action is that of decreasing the instability of the diabetes.

The studies here reported describe clinical experience using DBI in diabetic patients with especial attention to the last two actions above. In addition, two groups of patients presented the problem of the relatively more unstable diabetes of children (Pearlman, White) and another two groups comprised patients with non-diabetic complications upon whom unstable diabetes or poor regulation might have an adverse effect (Kleefield, Perkin). Finally, patients with severe and unstable diabetes were studied with respect to changes in blood electrolytes, with and without DBI therapy.

Presented at the Symposium on "A New Oral Hypoglycemic Agent, Phenformin (DBI)" in Houston on Feb. 6, 1959.

CASES AND METHODS

In all, these eight reports included a total of 280 diabetics, of whom *sixty-four were specified* as having the juvenile type of diabetes; an unspecified additional number probably had this type of diabetes also (table 1). All were ambulatory except for eleven hospitalized for tuberculosis (Kleefield) and six for metabolic studies (Perkin). Where an effect on instability or severity of diabetes was being studied, these factors were carefully defined, as were the criteria for clinical improvement (Kleefield, Perkin). The latter included blood and urine sugar determinations, as well as other clinical features, such as progress of complicating tuberculosis, electroencephalograms in patients with seizures,

TABLE 1

Author	Total number of patients	Number of juveniles
H. Dolger	100	Few
B. Greenhouse	30	Unknown
E. A. Kleefield	11	Unknown
W. Pearlman	57	14
F. S. Perkin	9	Unknown
M. Protas	29	12
R. S. Radding	6	Unknown
P. White	38	38
Total	280	64

and serial electrolyte determinations, depending on the study being reported.

RESULTS

Five discussants specifically reported on the reduction in insulin requirements of patients treated with a combination of DBI and insulin (Greenhouse, Kleefield, Pearlman, Perkin and White). DBI was given as a supplement to such patients when it could not entirely replace the insulin. Thirty-five of eighty-four patients (42 per cent) had a reduction in insulin needs of from 10 to 85 per cent as a result of the oral administration of 100-200 mg. DBI per day. Among an additional thirty patients, several were reported to have had similar reductions in insulin needs without detailed statistical evidence being furnished.

The same five investigators found a stabilizing effect of DBI upon "brittle diabetes" among 145 diabetic patients specifically studied for this purpose. The dose of DBI used was also 100-200 mg. per day in divided doses. Clinically, this most frequently took the form of the simultaneous absence or near absence of both glycosuria and hypoglycemic reactions in such groups of patients. In a small group, statistical evaluation showed that seventeen of twenty patients evidenced such an improvement.

When hypoglycemic reactions did occur during the adjunctive use of DBI with insulin, they were infrequent, usually mild, and never reached the stage of unconsciousness, in marked contrast to the frequency and severity of these reactions prior to the administration of DBI. The decrease in blood sugar variations corroborated these clinical observations. The absence of the usual hypoglycemic symptoms at low blood sugar levels was commented upon by two observers. Placebos were used by some as controls to establish the stabilizing effects of DBI.

These satisfactory features of DBI therapy were particularly noted by those dealing with childhood diabetes (Pearlman, White). In these patients, the duration of diabetes was shorter than in the adult groups, but the instability greater. Growth was normal in such children whether measured by the usual clinical criteria (Pearlman) or by the Wetzel grid (White). It was also stressed that such children could indulge in exercise and have greater latitude in meal scheduling with less danger of reaction (Pearlman).

Most authors observed that even under DBI therapy intercurrent bacterial or viral infections still adversely affect diabetes and increase its instability (Pearlman). Others reported an effect even upon this unsettling factor (White). Kleefield discussed the use of DBI in conjunction with insulin in eleven patients with tuberculosis and exceedingly unstable diabetes. Since the tuberculosis tends to add to the instability of the diabetes, and since it is clinically desirable for the healing of the tuberculosis to have as normal a blood sugar as possible, DBI was administered to such patients to minimize instability. In eight out of eleven of these patients, definite stabilization of the diabetes was effected.

A special group of unstable diabetic patients of the juvenile type complicated by seizures of central nervous system origin was studied by Perkin. All had electroencephalographic abnormalities, Grade III, and had been on Dilantin for extended periods of time. The latter drug decreased the frequency of seizures, which were sometimes mistaken for hypoglycemic reactions. However, the Dilantin did not affect the basic instability of the diabetes. Extensive experience had shown both the importance of preventing further cerebral damage by reducing the incidence of hypoglycemic reactions and the possibility of reversing mental changes already present as a result of such reactions. Accordingly, nine such patients were treated with DBI and insulin in an effort to stabilize the diabetes itself. A more stable blood sugar profile resulted, and the incidence of seizures decreased. In some patients, a parallel improvement in disposition, mental status and electroencephalogram resulted.

This beneficial effect of DBI was also noted by White in six children with seizures.

The previously reported additive effect of DBI and the arysulfonylureas in treatment of diabetes was confirmed by two investigators (Dolger, Pearlman). Dolger reported on such combined therapy in 100 maturity-onset diabetic patients, twenty-five with primary and seventy-five with secondary tolbutamide failure. The dose of tolbutamide had been 1.0 gm. per day and that of the DBI 50 mg. once daily. Satisfactory conversion of these patients from therapeutic failure on tolbutamide to success was obtained by the addition of DBI. Pearlman corroborated this finding in a smaller group of seven patients (included among his fifty-seven patients) with more conventional dosage levels of DBI (Pearlman) as had been reported previously.[2]

Gastrointestinal intolerance to the drug was noted in the present group of patients. Of seventy-seven patients reported in detail, ten (13 per cent) had to abandon therapy because of these symptoms (Greenhouse, Pearlman, Perkin). It should be noted that the incidence of significantly disturbing gastrointestinal symptoms was minimized in this series by the familiarity of these in-

vestigators with the drug and the policy of raising the dose slowly by small increments (25-50 mg. per day) over intervals of approximately a week each. Where the dose was only 50 mg. per day such symptoms were completely absent (Dolger). No toxic effects attributable to DBI were reported in any of these 280 patients.

Radding studied six unstable diabetics while in the process of regulation of DBI and insulin. In addition to blood and urine sugar determinations, blood sodium, chloride, CO_2, potassium, *p*H and lactic acid were measured over several days of change in regulation. Certain biochemical variations were encountered, but these were correlated solely with changes in blood sugar rather than with the DBI administration.

DISCUSSION

The reduction in dose of exogenous insulin by addition of DBI in insulin-treated diabetics has been previously reported.[2,3]

The reduction of instability of the diabetes in so-called "brittle" diabetes is a unique action of DBI. Historically, it was only after more evenly acting types of insulin replaced crystalline insulin that the presence of other sources of metabolic instability became more clearly evident in treated diabetics. Of these various causes for instability, the least understood has been the "innate" lability that DBI affects. Various of the present authors have commented on the experience that, once the innate variability is minimized or removed, the other sources of lability become more readily recognizable. These include menses, exercise, fever, infection, etc. When the latter are isolated in this manner, measures can be taken to control them by anticipatory changes in diet or insulin available to the patient.

It is of theoretical interest to know whether the "stabilizing" effect of DBI in "brittle" diabetes is identical with an insulin-sparing action of the drug. Where details are adequate for evaluation, it seems that the "stabilizing" effect of DBI follows significant insulin-sparing, but is not always identical with it.

Less controversial is the relationship between diabetic instability and the occurrence of hypoglycemia and the clinical well-being of a subject prone to seizures of central nervous system origin (Perkin). The same may be said for degree of diabetic control and healing of tuberculosis or other infections in a diabetic (Kleefield).

The confirmation of the ability of DBI to add to the action of sulfonylureas is important (Dolger, Perkin). This principle widens the scope of each type of drug and increases the per cent of patients who can be controlled entirely by oral therapy.[1]

SUMMARY

1. A total of 280 diabetic patients, including sixty-four with juvenile diabetes, were treated with phenformin.

2. Beneficial effects upon the diabetes in the form of reduced insulin needs, stabilization of "brittle" diabetes, and the facilitation of the action of the arylsulfonylurea drugs were reported.

3. The drug could be used with success in control of the lability of diabetes in the presence of, and during treatment of, tuberculosis.

4. Secondary benefits from stabilization of diabetes were observed in the clinical course of patients with seizures of central nervous system origin.

5. No toxic effects were reported.

6. Gastrointestinal side effects were minimized by the method of gradual increase in dose of the drug to its effective level.

SUMMARIO IN INTERLINGUA

Phenethylbiguanida in le Manipulation de Diabete Mellite

1. Un gruppo total de 280 diabeticos, incluse sexanta-quatro con diabete juvenil, esseva tractate con phenformina (DBI).

2. Es reportate effectos benefic super le diabete, in le forma de reducite requirimentos de insulina, le stabilisation de diabete "fragile", e le facilitation del action de drogas arylsulfonylureal.

3. Le droga poteva esser usate a bon successo in le domination del labilitate de diabete in le presentia o durante le tractamento de tuberculose.

4. Beneficios secundari resultante del stabilisation de diabete esseva observate in de patientes con convulsiones de origine in le systema nervose central.

5. Nulle effectos toxic esseva reportate.

6. Effectos lateral gastrointestinal esseva reducite al minimo per le methodo del augmento gradual del dosage.

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Symposium on "A New Oral Hypoglycemic Agent, Phenformin (DBI)"

General Summary

Alexander Marble, M.D., Boston

To summarize the available data and current views regarding β-phenethylbiguanide (DBI) is not an easy assignment. During the two days of this symposium, conflicting opinions were expressed about various aspects of the problem, particularly regarding the mode and site of action of the drug. In certain instances, as is often the case in the interpretation of scientific data, differing conclusions may be explained by the fact that the conditions of the studies have varied from project to project, from laboratory to laboratory and from clinic to clinic. Even in this group of experienced investigators, in the evaluation of findings one must take into account factors such as the type of experiment, e.g., whether in vitro or in vivo, the species of animal used, the tissues studied, the size of dose and route of administration of the drug, duration of the observations and many other conditions of study which may influence results.

There is general agreement that lowering of the blood sugar follows the oral or parenteral administration of DBI in almost all normal animals studied thus far. One exception, as yet unexplained, is that dogs are relatively unresponsive, exhibiting only slight and irregular falls in blood sugar. Ungar early reported that DBI had a hypoglycemic effect in rats, rabbits and monkeys previously made diabetic by means of alloxan. In many human diabetic patients, young and old, the giving of DBI by mouth produces a fall in blood sugar. There is much to suggest that the presence of insulin, of either endogenous or exogenous source, is essential for this hypoglycemic effect. Strangely enough, nondiabetic human subjects are not responsive to DBI.

Studies of toxicity. Following the giving of overly large doses of DBI to animals, death occurs in hypoglycemia before other changes can be observed (Volk, Ungar). When given to animals in effective hypoglycemic doses for periods up to six months, no toxic effects were noted either during life or on examination of tissues after sacrifice of the animals. When very large doses (100 mg./kg. twice daily) were given intravenously to rabbits, hyaline droplet formation and vacuolization of the proximal convoluted tubules of the kidneys were noted.

In diabetic patients Skillman found no liver damage as gauged by cephalin flocculation, bromsulfalein retention, alkaline phosphatase, prothrombin and serum transaminase tests. Studies of the peripheral blood gave no evidence of bone marrow damage.

Studies in vitro. The blood-sugar-lowering effect of DBI is easy to demonstrate. However, to elucidate the mode and site of action of the drug is a far more difficult matter. Various participants have described results obtained with the isolated rat diaphragm. The consensus was that, using such a preparation, the addition of DBI in concentrations of 0.1 to 0.4 mg. per ml. of incubation medium is followed by a decrease of glucose in the medium. However, this glucose apparently is not deposited as glycogen in the muscle such as occurs when insulin is used, but rather, oxygen consumption is inhibited and lactic acid formation is increased. The increase in glucose uptake is not accompanied by an increase in pentose space. DBI did not prevent glycogen formation by insulin in diaphragm experiments.

Using tissue homogenates and preparations of mitochondria, investigators have obtained results which appear to indicate that DBI interferes with some electron transfer mechanism and inhibits oxidative phosphorylation. In any case, the breakdown of glucose seems to proceed to a greater extent along the Embden-Meyerhof pathway, commonly spoken of as "anaerobic glycolysis," and to a less extent over the oxidative pathway of the Krebs cycle. Blocks in the oxidative pathways under

Presented at the Symposium on "A New Oral Hypoglycemic Agent, Phenformin (DBI)" in Houston on Feb. 6, 1959.

From the Joslin Clinic and the New England Deaconess Hospital, Boston, Massachusetts.

the influence of DBI have been postulated but there is disagreement as to the site of inhibition in the chain of respiratory enzymes. Reference was made by various participants to the published conclusions of Steiner and Williams[3] that the effect of DBI is at the cytochrome level in contrast to those of Wick and his associates,[2] who localized the effect on electron transport at the level between succinic dehydrogenase and reduced cytochrome C.

Studies in vivo. There was considerable discussion as to the applicability of results obtained in vitro to events in the living animal. Ungar in particular was of the opinion that the mode of action of DBI cannot be explained on the basis of findings in vitro. He stated that among various biguanides there is a lack of correlation between hypoglycemic effect and action in vitro. He stated further that the enzyme inhibitions found with DBI may be observed with the use of a large variety of drugs which have no hypoglycemic effect.

In diabetic subjects DBI produces, along with a fall in blood sugar, a rise in blood pyruvate and lactate. Pyruvate and lactate are apparently utilized since only small amounts are excreted in the urine. If the metabolites are produced in excess of muscle needs, such utilization probably is accomplished in the splanchnic circulation, presumably in the liver.

Bergen reported that DBI produced a fall in blood nonesterified fatty acids (NEFA).

From data derived from femoral arteriovenous glucose differences, Madison concluded that "either there is no increase in peripheral glucose utilization after DBI or that the increase occurs at a rate which cannot be detected by this experimental approach." On the other hand, from studies which included simultaneous determinations of blood glucose in the hepatic vein and femoral artery in a small series of patients, Tranquada inferred that the primary action of DBI is the stimulation of increased peripheral glucose utilization. His speculation was based on the finding of no significant change in the hepatic glucose output or oxygen consumption despite a fall in arterial glucose. He found no increase in arterial lactate and pyruvate levels; unfortunately it was not practicable to include a study of the venous blood sugar. Tranquada found the estimated hepatic blood flow (BSP method) to be increased by DBI.

Metabolic balance studies revealed no influence of DBI on the excretion of 17-hydroxycorticoids or 17-ketosteroids (Skillman, Fajans) and there was general agreement that DBI does not act by means of effect on the pituitary-adrenal axis (Bergen, Fajans, Skillman). DBI produced no effect on the excretion of nitrogen or potassium in the urine (Fajans).

Thus one must conclude that the exact site and mechanism of action of DBI are as yet not clear. However, the guideposts for future investigation have been put up and it is reasonable to hope and expect that workers attending this symposium will return to their respective laboratories with new ideas and plans for further study.

Clinical Studies. Turning to the clinical side, one finds general agreement that DBI exerts a hypoglycemic effect in a high percentage of diabetic patients of varying ages and durations of diabetes. In certain of these patients, presumably those whose pancreas is able to secrete some insulin, DBI may be used alone. In others, and particularly in children and those with the juvenile, unstable type of diabetes, DBI is effective if a basic dose of insulin, suited to the individual, is given. A high incidence of side effects consisting of anorexia, nausea, vomiting and diarrhea may be expected if daily doses are given which exceed (in adults) 150 to 200 mg. Smaller doses must be given to children and, furthermore, in any patient the dose must be suited to the individual. It is comforting to note that no evidence of acute or chronic toxicity on the liver, kidneys or bone marrow was reported during periods of observation extending well over two years.

Certain of the participants in the symposium made pleas for insistence on carefully conducted studies if these were to be the basis for conclusions as to drug efficacy. Some of the points made were: 1. Due regard should be paid to the effect of dietary restriction alone and the conditions of the studies should include adequate provision for furnishing a uniform diet. 2. Criteria as to control should be set up and observed. These include, among other items, levels of blood and urine sugar deemed necessary for classification of results as to good, fair and poor. In view of the well-established value of insulin, such criteria should be not only as strict but even stricter than for patients on insulin therapy alone. 3. The use of placebos often will aid in evaluation of efficacy of the drug. 4. Studies should be carried out over long periods of time with patients under close observation.

Other matters of clinical interest deserve mention. First, the development of acetonuria seen occasionally in patients on DBI has been brought up several times. There was general agreement that this is due to inadequate carbohydrate utilization and will disappear with increase in carbohydrate in the diet and perhaps increase in insulin dosage along with decrease in the dose of DBI. Second, the use of DBI in connection with

other oral hypoglycemic agents was described by certain participants. Success was claimed but obviously this field of activity demands further exploration before definite conclusions may be drawn.

A plea was made for careful control of diabetes as gauged not only by urine tests for sugar but also by blood sugar determinations made frequently. The renal threshold is elevated in many middle-aged and elderly diabetics, thus making urine sugar tests inadequate as a sole guide in treatment. Along this line, it was brought out that insulin remains the standard preparation for the treatment of diabetes and must be relied upon for its life- and health-saving properties in patients with severe diabetes, during ketoacidosis and at times of stress. One should make full and free use of available knowledge regarding the use of insulin as acquired over the past thirty-six or thirty-seven years. It is likely that if and when DBI is made available for general use, the greatest hazard will arise, as with other oral hypoglycemic agents, not from the drug itself but from its unwise use with patients who really need insulin.

CONCLUSIONS

What then is our collective opinion regarding phenformin at the end of this symposium? I believe I may summarize this as follows:

1. Phenformin is a biguanide which, taken by mouth, has a blood-sugar-lowering effect in certain diabetic patients of all ages.

2. In children and in adults with the unstable, juvenile type of diabetes, it usually must be combined with appropriate injections of insulin. It is doubtful if the drug is effective in the total absence of insulin, whether of endogenous or exogenous origin.

3. In hard-to-manage, unstable diabetes, it is said in many instances to smooth the course considerably, decreasing the tendency to symptomatic hypoglycemia. In such patients the reduction in insulin dose may amount to as much as 50 per cent of that required without phenformin. In diabetic children, phenformin should not be given without insulin lest general development and growth suffer.

4. Although side effects directed toward the gastrointestinal tract are common, especially with higher doses, no true toxicity has yet been encountered in human patients during more than two years' administration.

5. The mode and site of action of phenformin are still controversial. It appears, however, that its action differs from that of insulin and of the sulfonylureas. Following phenformin, glycolysis proceeds to a greater extent along the Embden-Meyerhof pathway ("anaerobic glycolysis") and to a less degree over the oxidative pathway of the Krebs cycle. Interference with some electron transfer mechanism with blocks in the oxidative pathways has been postulated.

6. Phenformin is a compound of great interest. Further studies are indicated, on the one hand, to clarify its mechanism of action, and on the other hand, to define its place in the management of diabetes.

SUMMARIO IN INTERLINGUA

Symposion Sur Un Nove Hypoglycemico Oral, Phenformina (DBI). *Summario del Processo Verbal*

1. DBI es un biguanida le qual, per via oral, reduce le sucro del sanguine in certe diabeticos de omne etates.

2. In juveniles e in adultos con le instabile typo juvenil de diabete, illo usualmente debe esser combinate con le appropriate injection de insulina. Il es dubitose que le droga es active in le absentia total de insulina, de origine endogene o exogene.

3. In casos difficile e instabile, illo pare frequentemente capace a render le curso del morbo plus lisie per reducer le tendentia a hypoglycemia symptomatic. In tal patientes, le reduction del dosage de insulina rendite possibile per DBI pote attinger 50 pro cento del requirimento sin DBI. In juveniles diabetic, DBI non deberea esser administrate sin insulina a causa del risco que le disveloppamento general e le crescentia suffre.

4. Ben que effectos lateral de orientation gastrointestinal es commun, specialmente con dosages plus alte, nulle ver toxicitate ha essite incontrate in patientes human in le curso de plus que duo annos de administration.

5. Le modo e le sito de action de DBI es ancora controverse. Il pare que su action differe ab illo de insulina e del sulfonylureas. Post le administration de DBI, le glycolyse seque plus extensemente le via de Embden-Meyerhof ("glycolyse anaerobie") e minus le via oxydative del cyclo de Krebs. Un interferentia in le un o le altere mechanismo de transferimento de electrones con blocage in le via oxydative ha essite postulate como un explication possibile.

6. DBI es un composito de grande interesse. Studios additional es indicate, de un latere, pro clarificar le mechanismo de su action e, del altere latere, pro definir su placia in le tractamento de diabeticos.

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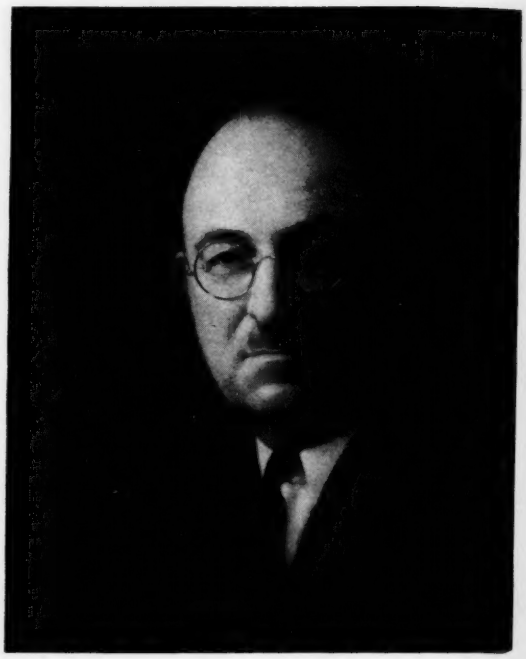
[2] Wick, A. N., Larson, E. R., and Serif, G. S.: A site of action of phenethylbiguanide—a hypoglycemic compound. J. Biol. Chem. 233:296, August, 1958.

Edward S. Dillon
1890-1959

Joseph T. Beardwood, Jr., M.D., Philadelphia



Edward Sanders Dillon was born in Woodbury, New Jersey, on March 21, 1890. He went to the William Penn Charter School and attended Princeton University. Here one of his extracurricular activities was the rowing crew and in later life he often referred to their reunions. After graduating in 1911, he went to Harvard Medical School from which he was graduated in 1916, when the first World War was in progress. He enlisted in the British Army and served as a Captain in the Royal Army Medical Corps in 1916, returning to Philadelphia to fulfill his internship in 1917-18 at the Pennsylvania Hospital. This, in turn, was followed by a year in the U. S. Army Medical Corps (1918-19) and at the end of the war he began his medical career in Philadelphia. Disregarding details, his medical life consisted of the following more or less concurrent activities. He was an attending physician at the Pennsylvania Hospital, where with Dr. Garfield Duncan he taught the use of insulin after its discovery in 1921. From 1923 until his retirement in 1956 he engaged in the private practice of medicine. In this he was associated with the late Dr. Orlando Petty for a while and diabetes was always his particular interest. Dr. Petty was on the staff of the Philadelphia General Hospital and this led to Dillon's third and perhaps greatest interest. At this hospital one of the first and most complete Metabolic Units was organized and in time Dr. Dillon became the chief of this division. Finally, he became Assistant Medical Director of the Penn Mutual Life Insurance Company. As one might expect with these manifold medical activities, conducted with ability, he was an Associate Professor of Diseases of Metabolism in the Graduate School of the University of Pennsylvania.

He was one of the founders of the Philadelphia Metabolic Association and a member of the American Diabetes Association from its inception. He served as its president in 1947-48. It was during his presidency that the Association expanded in several noteworthy respects, which included the publication of ADA FORECAST, an increase in the Affiliate Associations and the decision, realized a year later, to establish a central office for the American Diabetes Association. Dr. Dillon was a member of a number of medical societies, local and national, and his interest in fields of science outside of medicine was typified by his membership in the Rittenhouse Astronomy Society.

In 1923, he married Eugenia Vansant and their home was a delight to all their friends until her death in 1954. They had five children, a daughter and four sons, two of whom are physicians. In 1956 he married Mrs. Katherine S. Disbrow who survives him.

Ed Dillon was a man whose blunt manner often hid his friendly and even sensitive nature. He was a champion of the good and the right at all times and in the most practical applications of truth and right to the problems of daily life. From member to President and thereafter he served this Association faithfully and vigorously. His effort helped to bring us where we stand today, when we can still use his words: "We have high hopes for the success and the usefulness of the American Diabetes Association. It is still very young and new. In spirit and outlook we aim to keep it young."

We who mourn his passing may well strive to retain his forward thinking.

Luigi Zoja
1866-1959

Angelo Iannaccone, M.D., Florence, Italy

Prof. Luigi Zoja, an outstanding figure in modern Italian medicine and the leading diabetologist in Italy, died in Milan on June 10, 1959, at the age of ninety-three, still of a perfectly clear mind.

He was born in Pavia on Nov. 25, 1866. He attended the Medical School at the University of Pavia, where his father, Giovanni Zoja, was Professor of Anatomy, and graduated in 1889. Also in Pavia he frequented the Institute of Histology directed by the Nobel Prize winner, Camillo Golgi, and the Institute of Pathological Anatomy directed by Prof. Achille Monti. He then spent one year (1892) at the Institute of Physiology in Basel, Switzerland, studying analytical chemistry under G. v. Bunge. In 1895 he studied biochemistry in Berlin under H. Thierfelder.

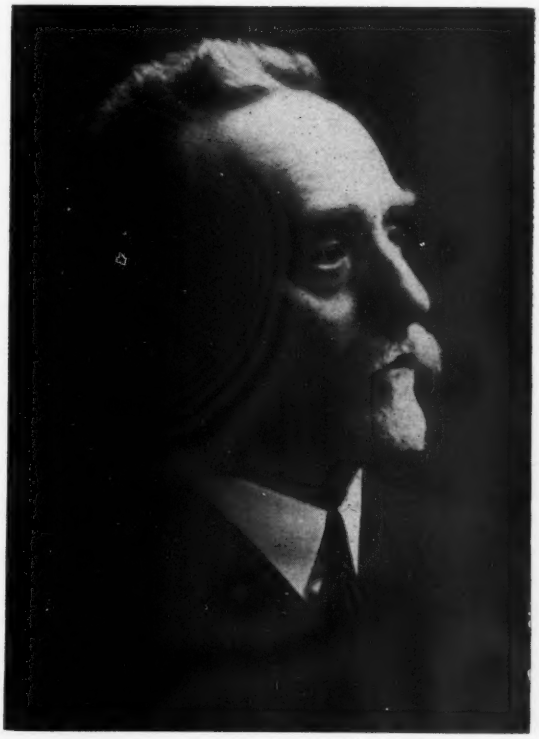
From 1893 to 1911 he was assistant to a distinguished Italian internist, Prof. Alberto Riva, at the University of Parma. In 1911 he was called to become Professor of Internal Medicine at the University of Sassari, Sardinia, where he remained until 1915, when he moved back to the University of Parma in the same academic capacity. In 1918 he was appointed Professor at his alma mater, the University of Pavia. In 1924, when the new University of Milan was founded, he accepted the professorship of internal medicine, and remained there until he reached his retiring age in 1937. The department of internal medicine in Milan had to be built up from the very beginning. Professor Zoja put all his experience, enthusiasm and authority into the new project. He was able to obtain large support from Government and private sources, and established a center of medical research which rapidly developed into one of the most active and best reputed Italian institutes for clinical investigation.

A brilliant mind, a very good and modest character, a continuous inspiration to those under him, he gave an example of hard work, strong moral qualities and scrupulous diligence in drawing conclusions from his observations and experiments. Many of his pupils have attained professorships at important universities: the late Adolfo Ferrata at Pavia, Antonio Gasbarrini at Bologna, Luigi Villa at Milan, Enrico Greppi at Florence, Ludovico Pontoni at Naples, Berel Salkind at Ekaterinoslav, Russia. Many others reached prominent positions in hospitals or research institutes.

His chief scientific merit is to have been one of the first to realize how great a contribution biochemistry and biophysics can give to clinical medicine, and to have been able to form a school where the bedside study of patients according to the old and fruitful tradition of Latin countries was successfully integrated with thorough investigation of the metabolic changes underlying or following the disease process.

His personal contributions extended into many fields of internal medicine and especially diseases of the liver and pancreas, bile pigment metabolism, and diabetes mellitus.

The results of his extensive studies on diabetes mellitus



Assistant Professor of Internal Medicine, Metabolic Unit, Institute for Occupational Medicine, University of Florence, Italy; Member of the American Diabetes Association.

were presented in a General Report to the Italian Society for Internal Medicine in 1931. He reaffirmed the central role of the pancreatic deficiency in the pathogenesis of diabetes mellitus, which then was still considered by some European schools as being of "pluriglandular" origin. He advocated for the diabetics a diet which should be as near to a normal diet as possible both from the point of view of calories as well as the proportion between proteins, fats, and carbohydrates. The general principle of the diet was to give not more than 1 gm. of proteins, not less than 3 gm. of carbohydrates, and from 1 to 1.5 gm. of fat per kilogram of body weight daily. The diet was varied according to the nutritional status of the patient, the conditions of the heart, vessels, kidney, and liver, and his physical activity. If necessary, insulin was added in amounts sufficient to insure complete utilization of ingested carbohydrates and keep the urine sugar-free. Determination of the sugar in the urine collected between meals and several blood sugar estimations during the twenty-four hours were found to be helpful in the treatment of "brittle" diabetes.

In October 1931 he established in Milan one of the first Italian diabetes clinics. From October 1931 to December 1938, 2,300 diabetic patients attended the clinic, which is still very active today. He made the suggestion that a visiting physician and nurse should take care of diabetic patients temporarily unable to attend the clinic, so that their treatment could be kept under close control, and that a special ward for diabetics should be set up in the City Hospital of Milan. He recommended that the poor should get insulin free or at a lower price.

He also founded a new journal, *Archivio italiano per lo studio della fisiopatologia e clinica del ricambio* (Italian Archives for the Study of Physiopathology and Diseases of Metabolism), where many Italian contributions in the field of diabetes mellitus are being published.

BOOK REVIEW

PREVENTIVE MEDICINE. *Principles of Prevention in the Occurrence and Progression of Disease. Edited by Herman E. Hilleboe, M.D., and Granville W. Larimore, M.D. $12.00, pp. 731, 59 figures, W. B. Saunders Company, Philadelphia, 1959.*

Preventive Medicine is a well written, well organized treatise on a difficult subject edited by two outstanding authorities of this field, Herman E. Hilleboe, M.D., Commissioner of Health for the State of New York, and Granville W. Larimore, M.D., his Deputy Commissioner. The concept of preventive medicine is constantly undergoing change. A few decades ago the only concern was with the problems of potable water, milk-borne disease, waste disposal and prophylactic measures against diseases usually of bacterial infection. A glance at the index of the forty-one chapters indicates the tremendous widening of scope this subject has undergone. The private practitioner must continually polish his skills in this field. Because of public demand he must be a preventive medical practitioner as well as a curative one. The public health physician does not compete with the private physician in practice, but complements him by enabling the private physician to practice a better quality of preventive medicine.

Preventive Medicine sets forth an approach to the subject for medical students, general practitioners, specialists and professional workers in official and voluntary health agencies. The main theme of the book distinguishes between the prevention of occurrence and a secondary prevention, a prevention of progression of disease. In the various chapters covering a wide variety of subjects, thirty-one notable authors, recognized authorities in their field, use material from their lectures, seminars and articles.

The chapter on "Screening Methods for Diabetes Mellitus" is excellently written by Frank W. Reynolds. The author points out the fact that diabetes, in children, is usually a severe and invariably symptom-producing disease, whereas in adults it is insidious and frequently asymptomatic in onset. The most likely candidates to be tested in a screening program are relatives of known diabetics, the overweight individual and those over forty years of age. Mothers of babies born with a birth weight of over eight pounds should be screened for diabetes mellitus. The author quotes unpublished data from Wilkerson et al., showing the relative sensitivity and specificity of a postprandial urine test as compared to a postprandial blood sugar determination. His tables indicate that it would be necessary to recheck ten persons to confirm the diagnosis of diabetes in one person, on the basis of the urine test alone, whereas with the blood sugar determination only two persons need rechecking. The author discusses the Clinitron method of doing mass blood sugar surveys. This method will probably be outdated by the new, more accurate Autoanalyzer method.

Preventive medicine, as the editors comment in their book, is to "outline a practical guide to physicians who are willing to incorporate preventive medicine as an integral part of their day by day practice."

ABSTRACTS

Altschul, Rudolph, and Smart, T. A. (Saskatoon, Canada): INFLUENCE OF WATER DEPRIVATION ON SERUM CHOLESTEROL IN RABBITS. Geriatrics *14*:461-64.

Depriving rabbits of drinking water for forty-eight hours is followed by a significant rise in serum cholesterol. The rise is not parallel to hemoconcentration as established by red blood cell counts and hematocrits but is greater and also more erratic. This seems to indicate that additional factors such as stress, decreased degradation or excretion may influence the changes of the serum cholesterol, which factors seem not to affect the red blood cell count or the hematocrit. S.S.

Anonymous (Committee on Government Operations, United States Senate, and Its Subcommittee on Reorganization and International Organizations, U.S. Government Printing Office, Washington, D.C.): DIABETES. Patterns of Incidence of Certain Diseases throughout the World 29-31, Nov. 9, 1959.

Diabetes is the eighth leading cause of death, and after glaucoma and cataract, the third leading cause of blindness. The incidence of diabetes in the United States was estimated early in 1959 at 1.5 million known diabetics, plus another 1.4 million persons who have the disease and do not know it. This degree of incidence is believed to be the highest of any country in the world. Other English-speaking countries with a high death rate are Canada, Australia and New Zealand. The death rate is lower in eastern and southern Europe, in Central and South America and Asia, and lowest of all in the underdeveloped countries. Of the European countries Denmark and Belgium reported higher rates than Austria and Germany. A chart is given that shows the striking contrast in incidence of the disease between the United States and Japan.

There is ample evidence of direct association of diabetes with abundant nutrition; and the association of the disease with obesity is well recognized. One study in the United States shows that about 85 per cent of those who have become diabetic at the age of forty or above are overweight. In some diabetics weight reduction diminishes the diabetic symptoms. During both World Wars striking decreases in the incidence and severity of diabetes accompanied loss of body weight in certain European countries. When food supplies increased after the war, so did the incidence of diabetes. Development of "substitutes" for insulin has been rapid since 1957 and today about half a million diabetics in the United States are using oral hypoglycemic compounds. E.W.S.

Ansell, G. B.; and Spanner, Sheila (M.R.C. Neuropsychiatric Res. Unit, Whitchurch Hosp., Cardiff, and Dept. of Exper. Psychiatry, Med. School, Birmingham, England): THE EFFECT OF INSULIN ON THE FORMATION OF PHOSPHORYLCHOLINE AND PHOSPHORYLETHANOLAMINE IN THE BRAIN. J. Neurochem. *4*:325-31, October 1959.

The uptake of labeled orthophosphate into brain phosphorylcholine in vivo after a short exchange period was approximately twice that into phosphorylethanolamine and this was paralleled by the uptake into the corresponding phospholipids. In insulin hypoglycemia there was a significant depression of the uptake of labeled orthophosphate into phosphorylethanolamine but not into phosphorylcholine. The uptake of labeled phosphate into both phosphatidyl choline and ethanolamine was significantly depressed. W.R.K.

Bänder, A. (Pharmacological Labs., Farbwerke Hoechst AG., Frankfurt/Main-Hoechst, Germany): THE MODE OF HYPOGLYCAEMIC ACTION OF THE SULPHONYLUREAS D-860 AND BZ-55. German M. Month. *4*:373-76, November 1959.

The mechanism of action of the sulfonylureas is considered to consist of a sensitization of glucose-sensitive receptors, which causes the release of insulin. The sulfonylurea derivatives stimulate the new formation of alpha and beta cells. They also release adrenalin and noradrenalin from the adrenal medulla. W.R.K.

Beavan, D. W.; Nelson, D. H.; Renold, A. E.; and Thorn, G. W. (Harvard Med. Sch., Boston, Mass.): DIABETES MELLITUS AND ADDISON'S DISEASE: A REPORT ON EIGHT PATIENTS AND A REVIEW OF FIFTY-FIVE CASES IN THE LITERATURE. New England J. M. *261*:443-54, Aug. 27, 1959.

Six previously unreported cases with established diagnosis of Addison's disease and diabetes mellitus are described with follow-up observations on two previously reported cases. The literature surveyed in fifty-five cases previously reported was reviewed together with the eight cases discussed here. S.S.

Bedrossian, Robert H. (Vancouver, Wash.): DIAGNOSIS OF DIABETES BY THE OPHTHALMOLOGIST. Am. J. Ophth. *48*: 843-44, December 1959.

The diagnosis of diabetes by an ophthalmologist in an otherwise unsuspecting patient usually impresses both the patient and the physician to whom the patient is referred for care. Such a diagnosis is most commonly made on the basis of the ophthalmoscopic examination and presents no problem since fasting blood sugars are elevated and glycosuria is present. Less certain is the diagnosis of diabetes in patients who complain primarily of fluctuating vision and have no pathologic condition of the fundus.

The ophthalmologist must show a high degree of suspicion in examining such patients. Unless he is certain of what he wants in the way of laboratory help, the diagnosis of early diabetes may be missed. To be satisfied with negative urine sugars or a fasting blood sugar is to give a false sense of security to many an early diabetic. A normal fasting blood sugar and sugar-free urine do not rule out diabetes.

Internists are relying less and less on fasting blood sugars and urinalysis to rule out diabetes. Many are advocating the use of the postprandial blood sugar as the single most diagnostic test. W.R.K.

Bloom, Arnold (Whittington Hosp., London, England): REMISSION IN DIABETES. Brit. M. J. 2:731-34, Oct. 17, 1959.

In 550 newly diagnosed diabetics, 184 (33.4 per cent) were adequately controlled by dietary restriction from the start, while a further 145 (26.3 per cent) were ultimately controlled by dietary restriction alone after temporary treatment with insulin or tolbutamide. The remaining 221 (40.2 per cent) needed permanent insulin or tolbutamide. The

dangers of unnecessary prolongation of insulin therapy are exemplified by two cases of posthypoglycemic encephalopathy. W.R.K.

Bradley, T. R.; and Nelson, J. F. (Dept. of Physiol., University of Melbourne, Melbourne, Australia): THE EFFECT OF ALLOXAN DIABETES ON THE METABOLIC ACTIVITY OF RAT MAMMARY GLAND TISSUE. Australian J. Exper. Biol. & M. Sc. 37:373-82, August 1959.

Alloxan diabetes results in loss of weight and considerable mortality in litters of lactating rats. Insulin replacement restores near normal litter growth rates and decreases litter death rate to zero.

Metabolism of rat mammary gland slices in vitro after alloxan treatment closely resembles that observed after hypophysectomy of lactating rats. Insulin replacement therapy in vivo corrects a biochemical lesion responsible for an abnormal production of lactic acid.

Diabetes renders mammary gland slices more sensitive to insulin in vitro, an opposite effect to that observed following hypophysectomy of lactating rats. W.R.K.

Broberger, O.; Jungner, I.; and Zetterström, R. (Pediat. Clin., Karolinska Sjukhuset, Karolinska Institutet, Stockholm; Pediat. Clin., University of Gothenburg, Gothenburg; and Hosp. for Epidemic Diseases, Stockholm, Sweden): STUDIES IN SPONTANEOUS HYPOGLYCEMIA IN CHILDHOOD: FAILURE TO INCREASE THE EPINEPHRINE SECRETION IN INSULIN-INDUCED HYPOGLYCEMIA. J. Pediat. 55:713-19, December 1959.

Three cases of idiopathic hypoglycemia in childhood with no increase in the epinephrine secretion in insulin-induced hypoglycemia are reported. The possibility of hypofunction of the adrenal medulla as the cause of the hypoglycemic attacks is discussed. This theory is supported by the fact that all three children have been completely free from symptoms after continuous ephedrine medication. W.R.K.

Bronstein, Howard D.; Kantrowitz, Paul A.; and Schaffner, Fenton (Dept. of Pediat., Path. & Med., Mount Sinai Hosp., New York, N. Y.): MARKED ENLARGEMENT OF THE LIVER AND TRANSIENT ASCITES ASSOCIATED WITH THE TREATMENT OF DIABETIC ACIDOSIS. New England J. Med. 261: 1314-18, Dec. 24, 1959.

A case report is presented of an instance of acidosis in juvenile diabetes. Following unusually large amounts of insulin therapy required for the control of the patient, acute hepatomegaly developed. It was felt that this condition was the result of rapid administration of very high doses of Regular Insulin, the presence of high blood sugar, and the deposition of large amounts of glycogen in the liver parenchyma. Hepatic sinusoidal obstruction and reduced activation of antidiuretic substances by the glycogen laden hepatic cell are suggested as an explanation for the transient ascites. The unusual clinical course in this case is presented as a possible complication in the treatment of diabetic acidosis. The authors suggest wider use of liver biopsy, with application of histochemical and electron microscopic technics to clarify further the pathologic physiology of hepatomegaly in diabetic acidosis. J.A.

Chain, E. B. (Depts. Biochem. and Chem. Microbiol., Istituto Superiore di Sanità, Rome, Italy): RECENT STUDIES ON CARBOHYDRATE METABOLISM. Brit. M. J. 2:709-19, Oct. 17, 1959.

The findings reported in this survey represent a part of the work on intermediate carbohydrate metabolism which has been in progress in the author's department during the past ten years.

The new technic of automatic quantitative radiochromatography constitutes a powerful tool for studies on intermediate metabolism and opens up new avenues not readily accessible by other methods. It is of particular importance for studies connected with the elucidation of the mode of action of drugs and hormones, but should also be of interest to the clinician as a possible tool to obtain, from bioptic samples of tissues, a picture of their functional biochemical state which may eventually prove of considerable value for diagnostic purposes and complement the histological microscopic methods at present used. W.R.K.

Cohen, Alan S.; Vance, Vernon K.; Runyan, John W., Jr.; and Hurwitz, David (The Thorndike Memorial Lab. & the Second & Fourth (Harvard) Med. Services, Boston City Hosp., the Dept. of Med., Harvard Med. School & the Diabetes Serv., Boston City Hosp., Boston, Mass.): DIABETIC ACIDOSIS: AN EVALUATION OF THE CAUSE, COURSE AND THERAPY OF SEVENTY-THREE CASES. Ann. Int. Med. 52:55-86, January 1960.

A detailed analysis is given by the authors of a large series of diabetics (fifty-two) treated for seventy-two instances of severe acidosis at a City Hospital from 1952 to 1955. There were only four deaths, all in patients over sixty-six, and all with complicating disease. Of the fifty-two patients, twenty-one were diagnosed as diabetic concomitant with the acidosis. The remainder were known diabetics, all poorly controlled, and most were severe. The precipitating factors in over half the patients were emotional instability (27 per cent) and infection (25 per cent).

Clinically 31 per cent had abdominal pain and tenderness, disappearing in twenty-four hours. The mental state was somewhat but not strictly related to elevation of the blood sugar or decrease in the carbon dioxide concentrating power.

In therapy, the authors stress the need for use of hypotonic solutions intravenously, oral potassium and vigorous insulin administration in the first few hours. They reinstituted long-acting insulin within the first twenty-four hours wherever possible. Although blood phosphorus levels were studied, no correlations could be found with it or its correction and the clinical state. They emphasize the advisability of avoidance of unnecessary or prolonged urinary catheterization. S.B.B.

Dekaban, Anatole S. (National Inst. of Neurological Diseases & Blindness, N.I.H., Bethesda, Md.): THE OUTCOME OF PREGNANCY IN DIABETIC WOMEN. II. ANALYSIS OF CLINICAL ABNORMALITIES AND PATHOLOGIC LESIONS IN OFFSPRING OF DIABETIC MOTHERS. J. Pediat. 55:767-76, December 1959.

This is a continuation of a study, the first part of which was reported in a previous issue, and is a further analysis of the same case material. This article is devoted to a study of the clinical abnormalities and pathologic lesions of offspring of diabetic mothers. Of 157 pregnancies in forty-eight diabetic women there were eighteen stillbirths, of which thirteen were full term and five premature. There were six abnormal offspring which is 3.8 per cent of all pregnancies, and 7.6 per cent of the seventy-nine surviving children as compared to 0.4 per cent in matched controls. Of the six abnormal surviving infants born to diabetic mothers, three had severe congenital malformations, two had mental deficiencies, and one

had sequelae of birth injuries and epilepsy. Postmortem examinations on sixteen infants who were stillborn or died during the neonatal period showed pulmonary hyaline membrane disease in eight, congenital malformations in three, cerebral birth injury in two and no positive findings in three. Factors possibly pertaining to increased fetal loss and increased rate of morbidity are discussed. R.L.J.

Farkas, T. G.; Ivory, R. F.; Cooperstein, S. J.; and Patterson, J. W. (Western Reserve Univ. Sch. of Med., Cleveland; and Vanderbilt Univ. Sch. of Med., Nashville, Tenn.): THE ROLE OF INSULIN IN LENS METABOLISM. Am. J. Ophth. *48*:394-97, November 1959.

Studies have shown that the glucose uptake of isolated rat lenses can be increased either by adrenalectomy or by insulin injection prior to removal of the lens. In both cases the increase can be demonstrated only if the liver is intact. The increase following adrenalectomy is of the same order of magnitude as that following insulin injection, and injection of insulin into an adrenalectomized animal does not further increase the glucose uptake. The removal of the other visceral organs has no effect. W.R.K.

Forsell, O. M.; and Palva, I. P. (Central Hosp. of N. Carelia, Joensuu, Finland): A RAPID METHOD FOR TRUE BLOOD GLUCOSE ESTIMATION. Scandinav. J. Clin. & Lab. Invest. *11*: 409, 1959.

The authors have adapted a colorimetric method based on the condensation of glucose and 2-aminobiphenyl. The forty-five minutes required for the condensation reaction at normal steam temperature has been reduced to ten minutes by use of a domestic pressure cooker. G.A.W.

Forsman, Olof; and Gemzell, Carl A. (Dept. of Med., Falu Hosp., Falu Dept. of Obst. & Gynec., Karolinska Hosp. & King Gustav V's Res. Inst., Stockholm, Sweden): PLASMA LEVELS OF GROWTH HORMONE IN PATIENTS WITH DIABETES MELLITUS, HYPERCHOLESTEROLAEMIA AND LIVER DISEASES. Acta endocrinol. *32*:480-90, December 1959.

The authors measured the plasma "growth-hormone-like" factor by its effect upon rat tibial epiphysis in fifty-one patients. The latter included forty human diabetics, seventeen with onset under age forty-one. Among these diabetics twenty-four had elevated levels, with a greater incidence in those with onset at a younger age and with nondiabetic complications. It was present in younger diabetics with retarded growth and absent in those with diabetic nephropathy. The latter was attributed to renal factors.

The hormone was also present in the plasma of some patients with cirrhosis of the liver, with or without diabetes, and two patients with familial hypercholesterolemia. S.B.B.

Franckson, J. R. M.; Conard, V.; and Bastenie, P. A. (Clinique Médicale-Université Libre de Bruxelles, Belgique): MEASUREMENT OF THE FREE GLUCOSE DIFFUSION SPACE IN MAN BY THE RAPID INTRAVENOUS GLUCOSE TOLERANCE TEST. Acta endocrinol. *32*:463-79, December 1959.

The authors have shown that it is possible to demonstrate by fast infusion of glucose that the glucose space is indeed equal to the extracellular compartment as demonstrated by use of C^{14} glucose or other substances such as thiocyanate.

The effect of exercise, the addition of exogenous and stimulation of production of endogenous insulin upon this test is to produce an expansion of the glucose space attributable to the separate phenomenon of cellular penetration. S.B.B.

Garnet, James D. (Pennsylvania Hosp., Philadelphia, Pa.): PREGNANCY COMPLICATED BY DIABETES. Am. J. Obst. & Gynec. 79:140-47, January 1960.

Pregnancy as a form of diabetogenic stress to the maternal pancreatic islet cells is discussed. The need for adoption of a simplified classification of the severity of diabetes based upon the additional obstetrical risk is emphasized. The highest perinatal fetal survival will be accomplished when well-controlled diabetic pregnancies are terminated between the thirty-sixth and the thirty-eighth week and earlier when complicated by recurrent ketosis, preeclampsia, hypertension, or renal disease. The total perinatal fetal mortality in this study is 13.6 per cent. Probably the most important factor for improvement of fetal salvage is the full understanding by both patients and physicians of the necessity for well-planned and complete prenatal care. W.R.K.

Gates, Edwin W. (Dept. of Med., Niagara Falls Memorial Hosp., Niagara Falls, N. Y.): MARRIAGE AND PARENTHOOD IN THE DIABETIC PATIENT. New York J. Med. *60*:233-36, Jan. 15, 1960.

The proposed marriage of a diabetic patient presents an opportunity to the physician to guide and prolong a marriage which, without his help, would not be successful. Diabetic patients can have healthy, nondiabetic children if only one partner has diabetes and the other partner has no familial or hereditary history of diabetes. Early counseling of patients as to marriage if carried out by all physicians could diminish the hereditary factor in diabetes. The thorough education of the diabetic and the nondiabetic partner regarding the proper care of diabetes must be carried out before marriage. This task that physicians must assume is not easy. It is only with true dedication to this problem that we can hope to justify completely the faith that the diabetic patient places in us. W.R.K.

Giles, Kenneth M.; and Harris, John E. (John E. Weeks Memorial Lab., Dept. of Ophth., Univ. of Oregon Med. Sch., Portland, Ore., and Dept. of Ophth., Univ. of Minn. Sch. of Med., Minneapolis, Minn.): THE ACCUMULATION OF C^{14} FROM UNIFORMLY LABELED GLUCOSE BY THE NORMAL AND DIABETIC RABBIT LENS. Am. J. Ophth. *48*:508-17, November 1959.

Recorded C^{14} accumulation by lenses decreased as lens weight increased. Insulin had no effect on C^{14} accumulation from uniformly labeled glucose by lenses from normal or diabetic animals. The anterior portion of both the lens capsule and lens substance accumulated more activity than did the corresponding posterior portions. Approximately 62 per cent of the accumulated activity in normal lenses and 84 per cent in lenses of diabetic animals migrated to a point corresponding to a stainable glucose spot on paper chromatography. Decapsulation resulted in a lowered glucose uptake by lenses of both normal and diabetic animals. Lenses (intact or decapsulated) from diabetics took up less glucose from the medium than did normal lenses. Addition of insulin to a decapsulated lens system increased its glucose uptake. DBI caused a marked increase in the glucose uptake of normal intact lenses. W.R.K.

Gittleman, I. F.; Pincus, J. B.; Schmertzler, E.; and Annecchiarico, F. (Dept. of Ped. & Div. Biochem., Jewish Hosp. of Brooklyn & Dept. of Ped., State Univ. of N. Y. Coll. Med., Downstate Med. Center, Brooklyn, N. Y.): DIABETES MELLITUS OR THE PREDIABETIC STATE IN THE MOTHER AND THE

NEONATE; EFFECTS ON THE HOMEOSTASIS OF CALCIUM, PHOSPHORUS, AND SOME OTHER ELECTROLYTES IN THE NEONATE ON THE FIRST DAY OF LIFE. A.M.A. J. Dis. Child. 98:342-49, September 1959.

Measurements of blood calcium, phosphorus, sodium, potassium, CO_2, chloride and glucose were performed on two groups of newborn infants, one group born to diabetic mothers and the other group born to mothers who were described as being "prediabetic." Most of the infants were delivered by cesarean section. The authors found an increased incidence of hypocalcemia on the first day of life in both groups of infants. The incidence of hypocalcemia in infants born to diabetic mothers in good control could be related to the type of delivery (cesarean section). Three mothers with uncontrolled diabetes had babies with hypocalcemia. The incidence of hypocalcemia was highest in the offspring of mothers in the prediabetic state, suggesting a metabolic disturbance. Mean serum phosphorus levels were slightly elevated in both groups of infants, but actual hyperphosphatemic levels were not often observed. Sodium, chloride, and CO_2 showed no change. Potassium was higher than normal, especially in hypocalcemic infants. Mean glucose levels were higher than those reported for normal infants. R.L.J.

Goodner, Charles J.; and Freinkel, Norbert (Dept. of Med., Harvard Med. School, Boston, Mass.): CARBOHYDRATE METABOLISM IN PREGNANCY: THE DEGRADATION OF INSULIN BY EXTRACTS OF MATERNAL AND FETAL STRUCTURES IN THE PREGNANT RAT. Endocrinology 65:957-67, December 1959.

The diabetogenic effect of pregnancy was studied by observing the insulin degrading capacities of cell-free extracts prepared from liver, placentas, and fetuses of pregnant rats. Insulin degradation per unit mass of placenta remained constant through pregnancy and comparable to that of maternal or control liver specimens. Insulin degradation per unit mass of fetus increased late in pregnancy to twice the level found in other tissues. The highest insulin degrading activity in the fetal tissues was found in the skin.

Attention is drawn to the importance of the conceptus in endocrine metabolism during pregnancy. The significance of the insulin degrading capacity of the rat fetus remains obscure since insulin does not traverse the placental barrier in this species. H.L.W.

Gorman, C. K.; and Weaver, J. A. (Metabolic Dept., Royal Victoria Hosp., Belfast, N. Ireland): LONG-TERM RESULTS IN TREATMENT OF DIABETES MELLITUS WITH SULFONYLUREAS. Brit. M. J. 2:1214-17, Dec. 5, 1959.

The results of the two-and-a-half-year study of the control of 291 diabetic patients on oral therapy with tolbutamide is summarized. The findings of these investigators with regard to the type of patient suitable for treatment are in agreement with previous studies of other workers. Intravenous glucose tolerance tests indicate that the ability of the diabetic to utilize carbohydrates has not been adversely influenced by prolonged tolbutamide treatment. However, there was no clear-cut indication that the ability to utilize carbohydrate had been improved in a significant number of treated patients. It was also observed that there is less responsiveness in males to tolbutamide therapy than in females. J.A.

Goto, Y.; Umiie, Y.; Kato, J.; and Sasaki, Y. (Tohoku Univ. Hosp., Sendai, Japan): ACETYLATION OF SULFONAMIDE AND PARA-AMINO-BENZOIC ACID IN DIABETIC PATIENTS. New England J. Med. 261:424-30, Aug. 27, 1959.

Previous authors have found that metabolic acetylation is impaired in experimental diabetes. The present study was undertaken to ascertain whether such disturbances of acetylation occur in diabetic patients. This study shows that no significant impairment of acetylation is present in diabetes mellitus. S.S.

Hagedorn, H. C. (Gentofte, Denmark): CASES OF DIABETES OF LONG DURATION. New England J. Med. 261:442-43, Aug. 27, 1959.

A follow-up study of the first eight Danish diabetic patients treated with insulin in 1923 has been made. One died within the first two days of insulin treatment. One died twenty-two years later of cancer at seventy-four years of age. One died of coronary occlusion at seventy-two years of age, twenty-eight years after the beginning of insulin treatment. Five patients are still alive with an average duration of diabetes of forty-one years and insulin treatment of thirty-six years. Four of these have normal vision. One of them has been operated upon for cataract, two of them have minimal retinal lesions and the fifth is living abroad and is known to have impaired vision but is still leading an active life. S.S.

Harris, Robert H.; and Taylor, Fred, Jr. (Torrance Memorial Hosp., Long Beach, Calif.): PEMPHIGUS VULGARIS AND DIABETES MELLITUS: A CASE REPORT. A. M. A. Arch. Dermat. 80:442, October 1959.

A case of pemphigus vulgaris and diabetes mellitus was treated successfully with dexamethasone, a new variation of prednisolone, with no difficulty in the control of the diabetic condition. W.R.K.

Haunz, E. A. (Dept. of Int. Med., Grand Forks Clin., and Univ. of N. Dak. Sch. of Med., Grand Forks, N. Dak.): INSULIN, SULFONYLUREAS AND THE DIABETIC PATIENT. GP 19:124-31, April 1959.

The "middle of the road" policy toward reasonable control of diabetes, in which extreme hyperglycemia, insulin reactions and glycosuria are avoided as far as possible, is expressed in this article. Severity of diabetes is determined by the age and weight of the patient, duration and stability of the disease, and treatment is prescribed accordingly. Restriction of sugar alone is advised for mild cases, and a measured diet for severe cases. Insulin or sulfonylurea agents are used if the patient is markedly hyperglycemic or symptomatic; also if he is less than sixteen years of age. The ideal candidate for oral therapy is a patient who is more than forty years of age, who has maturity-onset diabetes and takes less than 40 units of insulin. The drugs are contraindicated in unstable cases, liver disease, juvenile cases or those with complications. Although chlorpropamide is more potent than other agents, it is also more toxic. Lente insulin is preferred to other types. Experimental use of both insulin and sulfonylureas is not recommended. A.R.C., JR.

Hawkins, R. D.; Ashworth, M. A.; Schacter, H.; and Haist, R. E. (Toronto, Canada): EFFECT OF CONTINUOUS GLUCOSE INFUSION ON THE GLUCOSE-SIX-PHOSPHATASE ACTIVITY OF THE LIVER IN RATS. New England J. Med. 261:434-37, Aug. 27, 1959.

The continuous infusion of glucose (0.8 ml. per hour of a solution containing 50 gm. per 100 ml.) caused the depression of hepatic glucose-6-phosphatase activity that was maximal

seventy-two hours after the infusion was started. The infusion of glucose into alloxan-diabetic rats with greatly reduced functional islet tissue brought about no significant change in the elevated level of hepatic glucose-6-phosphatase. In rats, in which a deficiency of islet function was temporarily induced by a four-week period of daily insulin injections, glucose was relatively ineffective in depressing glucose-6-phosphatase activity during the early stages after insulin withdrawal. In contrast, when the time following insulin withdrawal had increased by four to seven days, the ability of glucose to depress hepatic glucose-6-phosphatase activity returned. The depression of glucose-6-phosphatase activity of the liver by glucose infusion thus seems to be dependent upon islet activity and availability of insulin. S.S.

Ide, Mituki (Dept. of Physiol., Nagasaki Univ. Sch. of Med., Nagasaki, Japan): EFFECT OF INSULIN HYPOGLYCEMIA ON THE NORADRENALINE AND ADRENALINE CONTENTS OF THE SUPRARENAL GLAND IN CATS. Kumamoto M. J. *12*: 258-63, Sept. 30, 1959.

Some investigations were made as to the noradrenalin content in the suprarenal gland of cats. All investigators found that the relative amount of noradrenalin was comparatively greater in the cat's suprarenal gland. In cats the adrenalin content of the suprarenal gland was strikingly reduced by the administration of insulin in a dose of either 2 units or 5 units. However, the alterations of noradrenalin content were less in comparison with those of adrenalin content. Thus, the fact that insulin causes a marked decrease of adrenalin content in the suprarenal gland may indicate that the state of hypoglycemia needs an acceleration of adrenalin secretion rather than that of noradrenalin secretion. W.R.K.

Ikkos, Denis; Luft, Rolf; and Gemzell, Carl-Axel (Dept. of Endocrinol. & Dept. of Obst. & Gynec., Karolinska Sjukhuset, Stockholm, Sweden): THE EFFECT OF HUMAN GROWTH HORMONE IN MAN. Acta endocrinol. *32*:341-61, November 1959.

Small doses of human pituitary growth hormone, 10 mg. per day, produced decreased carbohydrate tolerance (by intravenous glucose tolerance test) and ketonuria within five days in four nondiabetic adults. One had been hypophysectomized. No hyperglycemia or glycosuria was induced.

Other metabolic changes of significance were also observed: sodium, potassium, chloride, nitrogen, phosphorus and water were retained, and by contrast, calcium was lost. The anabolic effect upon nitrogen metabolism was accompanied by a decrease in blood nonprotein nitrogen but there were no electrolyte changes in the blood. S.B.B.

Kekwick, A.; Pawan, G. L. S.; and Chalmers, T. M. (Dept. of Med., Middlesex Hosp. Med. Sch., London, W.1, England): RESISTANCE TO KETOSIS IN OBESE SUBJECTS. Lancet *2*: 1157-59, Dec. 26, 1959.

In order to test the utilization of "ketone bodies" six obese and six nonobese subjects were given intravenously 30 mg. sodium beta hydroxybutyrate/kg. body weight. Blood ketones were followed for 300 minutes. Butyrate distribution was in total body water and decline in blood ketones suggests equal rate of utilization in both obese and nonobese subjects.

In all subjects less than 1 per cent injected "ketone" was recovered in urine. It was concluded that resistance of the obese to ketosis is not relative to rate of utilization.

Subjects on high fat (90 per cent) diet gave pronounced ketosis in nonobese in two to four days while no marked ketosis was observed in obese subjects up to twelve days on diet. The nonobese excreted more nitrogen per day and ran lower blood sugars. It is presumed that resistance of obese to ketosis is due to decreased rate of ketone body production. Since blood glucose was higher in obese it is suggested that there may be a net conversion of fat to carbohydrate in this group. J.A.

Kinsell, Lawrence W.; Walker, Geoffrey; Michaels, Deurel D.; and Olson, Florence E. (Oakland, Calif., Inst. for Metab. Res., Hyland Alameda County Hosp., Oakland, Calif.): DIETARY FATS IN THE DIABETIC PATIENT. New England J. Med. *261*:431-34, Aug. 27, 1959.

A dietary approach to the problems of vascular disease in the diabetic patient is outlined. It is the authors' impression that in some cases the substitution of a polyunsaturated fat for mono-unsaturated fat results not only in a decrease of plasma lipids and in some cases apparent improvement in existing retinopathy and possible nephropathy but also in a significant improvement in diabetic management in terms of blood sugar levels on a regimen in which total protein, carbohydrate and calories remained unchanged. Their impression is favorable regarding the desirability of using diets containing relatively or absolutely large amounts of polyunsaturated fats in the management of diabetic patients with the hope that one may prevent the vascular complications or prevent the progression of vascular disease if already present.

A diet containing relatively large amounts of fat particularly rich in polyunsaturated fatty acids will of necessity derive most of its fats from vegetable sources and will largely exclude meat, dairy fats and eggs. It has a low degree of acceptance in terms of the average American palate. A high fat diet, in which perhaps two thirds of the fat is derived from predominantly polyunsaturated sources and a third from predominantly saturated sources (meat, eggs and dairy fat) will be much more acceptable than the preceding diet and in most patients will be associated with reasonably low plasma lipids. To be most effective, such a diet will emphasize fish and to some degree fowl at the expense of other animal protein and fat. S.S.

Knowles, Harvey C., Jr. (Metabolism Lab., Dept. of Int. Med., Cincinnati Coll. of Med., Cincinnati, Ohio): MANAGEMENT OF THE ADOLESCENT DIABETIC PATIENT. Postgrad. Med. *26*:766-72, December 1959.

The author discusses the management of the adolescent diabetic patient from personal experience and also gives a brief review of the literature. He underlines the greater tendency to ketosis in adolescent as compared with adult diabetics, and emphasizes the role of carbohydrate deprivation in the development of this condition in youth. Accordingly, in this age group glucose may be used in its therapy from the start. He also discusses the importance of the psychological aspects of the handling of juvenile diabetics. S.B.B.

Koenig, Elizabeth (National Institutes of Health, U. S. Dept. of Health, Education, and Welfare, Washington, D.C.): MATERNAL DISORDERS RELATED TO FETAL STRESS, PERINATAL DEATH AND CONGENITAL DEFECTS. Public Health Service Publication No. 669, Public Health Bibliography Series No. 25, 1959.

This thirty-three-page bibliography was compiled by the Reference Librarian of the National Institutes of Health Li-

brary, Division of Research Services. Of 413 titles related to the problems indicated in the title, fifty refer to those of the diabetic mother and her offspring. The bibliography is selective and includes only studies in the English language during the years 1952-58. This reference source focuses attention on recent studies and provides material for further investigations in these problems. A limited number of copies is available from the Library, National Institutes of Health, Bethesda 14, Maryland. Additional copies may be purchased from the U. S. Government Printing Office for fifteen cents per copy. E.W.S.

Lamar, Carlos P. (Dept. of Int. Med., Jackson Mem. Hosp., Miami, and Doctors Hosp., Coral Gables, Fla.): COMPARATIVE ORAL GLUCOSE AND FRUCTOSE TOLERANCE TESTS IN NORMAL SUBJECTS AND IN DIABETIC PATIENTS. J. Florida M. A. *46*:180-86, August 1959.

Failure of ingested fructose to relieve the signs and symptoms of hypoglycemic reactions in diabetic patients receiving insulin is presented as presumptive evidence that most of the ingested fructose is absorbed into the human blood stream as such and is not converted to glucose by the intestinal mucosa as has been generally believed. Apart from its significance as an emergency stabilizing factor of the blood sugar level, the importance of the role played by the conversion of either glucose or fructose to glycogen has probably been overemphasized. A discussion of the current theories of carbohydrate metabolism offers a possible explanation of the role of fructose as a major source of caloric energy in the diabetic patient with a possible reduction in the need for supplementary exogenous insulin. W.R.K.

Lazarow, Arnold; and Treibergs, Biurta (Univ. of Minn. Med. Sch., Minneapolis, Minn.): THE EFFECTS OF LONG-TERM ADMINISTRATION OF TOLBUTAMIDE IN ALLOXAN SUBDIABETIC RATS. New England J. Med. *261*:417-23, Aug. 27, 1959.

A group of rats, made subdiabetic by the use of alloxan, has impaired glucose tolerance and, when fed ad libitum, does not exhibit hyperglycemia or glycosuria. Both their fasting and postprandial blood sugar values are within normal limits. But, according to studies made in this laboratory, subdiabetic rats, unlike normals, appear to be more susceptible to certain diabetogenic agents. Tolbutamide, administered by stomach tube twice daily to subdiabetic rats in doses of 500 mg. per kg. of body weight per day, was observed to impair the tolerance of glucose; this impairment persisted throughout the twelve-month period of administration. Tolbutamide administered to subdiabetic rats in doses of 200 mg. per kg. per day produced less changes in the glucose tolerance. Even in the normal rat, prolonged administration likewise impaired the tolerance to glucose. The administration of the drug did not convert subdiabetes in rats into frank diabetes. The amelioration of the diabetic state previously observed in untreated animals during the second year of the disease was likewise observed in rats during tolbutamide treatment (500 mg. per kg. of body weight per day). S.S.

Leonards, J. R.; and Martin, F. I. R. (Western Reserve Univ., Cleveland, Ohio): INSULIN INSENSITIVITY. New England J. Med. *261*:68-70, July 9, 1959.

This is a report of a case of moderate insulin resistance with a high concentration of insulin-like activity demonstrable in the peripheral blood. The patient was a seventy-year-old female known to be diabetic for three years. She was semicomatose when hospitalized, with signs of a recent right-sided hemiparesis. Serum acetone was 30 mg. per 100 ml. and blood sugar 280 mg. per 100 ml. Diabetes was controlled with 380 units of Crystalline Insulin and the urine was clear after twenty-four hours. However, on the third hospital day, the fasting blood sugar was 273 mg. per cent and during the next week, glycosuria steadily increased, the fasting blood sugar rising to 465 mg. per cent, despite daily increases of insulin to a dose of 200 units (140 units of Protamine Zinc Insulin and 60 units of Crystalline Insulin) by the eleventh day. Clinically, the patient had improved, was ambulatory, afebrile, with no evidence of infection but with an almost complete right hemiplegia with some emotional lability. On the twelfth day, acetonuria was noted. The fasting blood sugar was 525 mg. per 100 ml. BUN was 22 mg. per 100 ml. and an additional dose of 200 units of Crystalline Insulin was given over the next eight hours; and on the next two days, because of glycosuria and persistent acetonuria, a total of 500 and 560 units of Crystalline Insulin was administered respectively. Over the next seven days, the insulin dose was gradually reduced to 200 units per day of NPH and Crystalline Insulin; the fractional urine tests remained almost clear of sugar, with fasting blood sugars of between 180 and 220 mg. per cent. On the twenty-fifth day, the patient had a severe episode of hypoglycemia and the insulin dose was reduced to 100 units per day and then as hypoglycemia persisted the dosage was further reduced gradually to 30 units of NPH insulin daily. For the last three months, good control had been achieved on this dose with a fasting blood sugar of between 100 and 140 mg. per 100 ml.

Insulin-like activity was assayed by the method of Martin et al., which measures the in vitro stimulation of the production of carbon dioxide from glucose-1 C^{14} in the rat epididymal fat pad. In this case, owing to the large amount of insulin-like activity present, the serum was diluted two hundred and fifty times to bring the degree of stimulation within the range of the insulin standards in use. The insulin-like activity of this patient's serum is 0.125 units per ml. (100 times greater than the highest value found in normal persons).

This patient's insulin resistance lasted for several weeks and terminated abruptly. The development of insulin resistance shortly after a cerebral thrombosis is of interest. In laboratory animals damage to the hypothalamus or brain stem can result in an elevated blood sugar. In addition, there is a clinical association between cerebral damage and hyperglycemia and there have been reports of diabetes occurring in association with hypothalamic tumors. S.S.

Logothetopoulos, J.; Sharma, D. B.; Salter, J. M.; and Best, C. H. (Toronto, Canada): "METAGLUCAGON DIABETES" IN RABBITS. New England J. Med. *261*:423-26, Aug. 27, 1959.

Nine albino rabbits were injected with 2 mg. of glucagon per kg. of body weight. Treatment began on the third postnatal day and continued for five or six months. Four of the animals showed only transient hyperglycemia after each injection. The other five animals became diabetic. Glucagon treatment was stopped after six months in three of the rabbits. The intensity of the diabetes in one of these animals remained unchanged for twenty days and then diminished. The second animal was diabetic and was killed for histologic studies on the thirtieth day. The third animal was still diabetic forty

days after the cessation of glucagon administration. Glucagon treatment of the remaining two rabbits is being continued. Histologic examination of the pancreases revealed extensive atrophy of the alpha cells in three of the glucagon-treated animals that were killed. Hypertrophy and hyperplasia of the beta cells without glycogen deposition were found in the two animals resistant to the diabetogenic action of the glucagon. The density of the islet tissue was reduced and there was massive glycogen infiltration of the beta cells of the pancreas of the one diabetic animal. Many more observations must be made before the complete picture of "metaglucagon diabetes" can be resolved. S.S.

Luft, Rolf; Ikkos, Denis; Gemzell, Carl-Axel; Olivecrona, Herbert (Dept. of Endocrinol. & Dept. of Obst. & Gynec., Karolinska Sjukhuset, Dept. of Neurosurgery Serafimerlasarettet, Stockholm, Sweden): THE EFFECT OF HUMAN GROWTH HORMONE IN HYPOPHYSECTOMIZED HUMAN DIABETIC SUBJECTS. Acta endocrinol. *32*:330-40, November 1959.

Purified human pituitary growth hormone had a distinct effect in increasing blood and urine sugar and ketonuria in three human juvenile diabetics who had undergone hypophysectomy. The effect was induced by a dose of 10 mg. per day and occurred within twenty-four hours in two, and five days in the third. In two of the subjects the administration of growth hormone had to be interrupted after the second day. In one patient, the one with metabolic studies, significant nitrogen retention was not observed. S.B.B.

McGavack, T. H. (V.A. Center, Martinsburg, W. Va.): A CRITICAL APPRAISAL OF ORAL HYPOGLYCEMIC AGENTS. J. Am. Geriatrics Soc. *9*:681-83, September 1959.

The author stresses that none of the currently available oral hypoglycemic agents is a substitute for insulin. The author asserts that all of them act upon both the pancreas and the liver but the nature of the activity is still obscured. They are useful in a small percentage of diabetics of the so-called "stable" type who require both insulin and a well-controlled diet to maintain optimal control. The long-term effects on the capacity of the pancreas to produce insulin and on the enzyme systems of the liver concerned with carbohydrate metabolism are not yet known. S.S.

Munkner, C. (Second Univ. Clin. of Int. Med., Kommunehospitalet, Aarhus, Denmark): FASTING CONCENTRATIONS OF NONESTERIFIED FATTY ACIDS IN DIABETIC AND NONDIABETIC PLASMA AND DIURNAL VARIATIONS IN NORMAL SUBJECTS. Scandinav. J. Clin. & Lab. Invest. *11*:388-93, 1959.

Studies on the procedure of securing blood samples for determining the plasma concentration of nonesterified fatty acids (NEFA) have been made using the method of Dole (J. Clin. Invest. *35*:150, 1956). It is found that prolonged standing, even at 0-4° C., and the use of even very small amounts of heparin to prevent clotting result in considerably increased values. The average NEFA is increased in both obese and nonobese diabetic patients. Day-to-day variations of 10 to 30 per cent are found in the NEFA levels of fasting human subjects. G.A.W.

Penttilä, I. M.; and Pöllänen, L. O. (Dept. of Med. Chem., Univ. of Helsinki, Finland): EFFECT OF INSULIN AND TOLBUTAMIDE ON BLOOD CITRIC ACID IN RABBITS. Scandinav. J. Clin. & Lab. Invest. *11*:322-25, 1959.

A comparison of the effects of insulin (0.5 units/kg. of body weight, subcutaneously) and of tolbutamide (100 mg./kg. of body weight, subcutaneously) on the blood citric acid level in young (six months) and old (fifty-four months) female rabbits has been made. The citric acid level was considerably higher in the young than in the old rabbits. Insulin caused a prompt fall in blood citric acid level in the young rabbits, but only a slow decrease after two hours in the old ones. Tolbutamide caused an initial increase in the blood citric acid of both old and young rabbits followed by a decrease in each case. In the young rabbits this decrease reached subnormal values within four hours. G.A.W.

Reynolds, Frank W.; Fons, Anthony L., III; and Pennisi, J. B. (Albany, N. Y.): ROUTINE TESTS FOR DIABETES MELLITUS AMONG ADULT HOSPITAL PATIENTS. Geriatrics *14*:784-88, December 1959.

Among a sample of 1,000 consecutive admissions to a large general hospital, the random urine of 981 patients was tested for glucose, and fasting blood sugars of 810 patients were determined. Routine urinalysis detected sixteen persons or 1.6 per cent in whom diabetes was suspected and routine blood sugar tests revealed fifty-seven persons or 7.3 per cent in whom diabetes was probable. The suggested screening tests were not followed up in approximately 40 per cent of the cases and routine follow-up left much to be desired. Routine testing for diabetes on hospital patients is an effective screening mechanism but it requires proper follow-up to become an effective case-finding device. S.S.

Riggs, Byron L., Jr.; and Randall, Raymond V. (Mayo Foundation and Mayo Clin., Rochester, Minn.): CLINICS ON ENDOCRINE AND METABOLIC DISEASES. II. DIABETES INSIPIDUS AND DIABETES MELLITUS: REPORT OF CASE. Proc. Staff Meet. Mayo Clin. *35*:30-33, Jan. 20, 1960.

A case of co-existing diabetes insipidus and diabetes mellitus has been reported. The diabetes insipidus apparently was caused by metastatic spread from a carcinoma of the breast. The combination of diabetes insipidus and diabetes mellitus has been noted in only seven of the more than 2,250,000 patients studied at the Mayo Clinic. W.R.K.

Swartwout, J. R.; Birch, Herbert W.; and Pailet, Max (Dept. of Obst. and Gynec., Tulane Univ. Sch. of Med., New Orleans, La.): GLUCOSE TOLERANCE TEST AND VULVITIS. Obst. & Gynec. *15*:131-33, February 1960.

Vulvitis in this clinic group has a high correlation with undiagnosed diabetes mellitus and abnormal carbohydrate metabolism. Fasting blood sugar and urinary sugar determinations alone are inadequate for the detection of diabetes mellitus in this group. All patients with generalized vulvitis should have a complete glucose tolerance test. W.R.K.

Walker, Robert S.; and Linton, Adam L. (Law Hosp., Carluke, Scotland): OBSERVATIONS ON THE TREATMENT OF YOUNG DIABETICS WITH AN ORAL DRUG (PHENETHYLBIGUANIDE). Acta endocrinol. *32*:491-96, December 1959.

This is a report on the treatment with phenformin (DBI) of thirty-one "juvenile" diabetics—unstable diabetics with onset of disease under age forty (average, twenty-one years). Successful replacement or avoidance of insulin was accomplished in ten and partial replacement in another fifteen patients (an average of 50 per cent). Of the latter, most patients demonstrated an increase in stability of regulation.

Gastrointestinal intolerance and tendency to ketosis, both detectable and remediable, were the only side effects noted. S.B.B.

ORGANIZATION SECTION

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20th Annual Meeting
American Diabetes Association
June 11-12, Deauville Hotel, Miami Beach, Florida

Priscilla White, M.D., will deliver the Banting Memorial Lecture during the Scientific Session Saturday morning, June 11. Her subject will be "Childhood Diabetes — Its Course, and Influence upon the Second and Third Generations." Dr. White is associated with the Joslin Clinic and the New England Deaconess Hospital, Boston, Massachusetts.

BANQUET HIGHLIGHTS

- The Banting Medal will be presented to Dr. White at the Annual Banquet of the Association on Saturday evening, June 11. J. B. Collip, Ph.D., M.D., D.Sc., Dean of Medicine, Faculty of Medicine, The University of Western Ontario, London, Ontario, Canada, also will be awarded the Banting Medal.
- Deaconess Maude Behrman of Atlantic City will offer the Invocation.
- Francis D. W. Lukens, M.D., Philadelphia, will deliver the Address of the President.
- Warren J. Wisby, Associate Professor of Marine Biology, The Marine Laboratory, University of Miami, Miami, Florida, will speak on "Social Behavior in Fishes."
- The 1960 Lilly Award will be presented to Albert E. Renold, M.D. The award, supported annually by Eli Lilly and Company, consists of $1,000 and a medal. Its purpose is to recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality. Dr. Renold is Assistant Professor of Medicine, Harvard Medical School; Director, Baker Clinic Research Laboratory, New England Deaconess Hospital; and Associate in Medicine (Biochemistry), Peter Bent Brigham Hospital, Boston, Massachusetts.
- The Annual Citation to an outstanding layman will be awarded to Miss Edith Jenkins of the Indianapolis Diabetes Association by Maurice Protas, M.D., Chairman of the Assembly of Delegates of the ADA.

SCIENTIFIC SESSIONS

Five Scientific Sessions have been scheduled this year, in place of the customary three. There will be Saturday morning and afternoon Sessions; two concurrent Sessions — one experimental and the other clinical — on Sunday morning; and a final Session on Sunday afternoon, June 12. Forty-one papers will be presented and twenty-one others will be included in the program by title. Blair Holcomb, M.D., is Chairman of the Committee on Scientific Programs, and Franklin B. Peck, Sr., M.D., is Vice Chairman. Members of the Committee include James Ashmore, Ph.D.; T. S. Danowski, M.D.; Irving Graef, M.D.; and Harvey C. Knowles, Jr., M.D.

- A special summary of the Symposium on Retinopathy, which was held in Haddonfield, New Jersey, May 1-2, by the National Institute of Neurological Diseases and Blindness,

will be presented by the Symposium Chairman, Irving H. Leopold, M.D. Dr. Leopold is Chairman, Department of Ophthalmology, The Graduate School of Medicine, University of Pennsylvania, Philadelphia.

- Although a joint meeting will not be held with The Endocrine Society this year, arrangements again have been made so that Endocrine Society members may attend the Scientific Sessions of the ADA, and ADA members may attend, without charge, sessions on diabetes of The Endocrine Society at the Eden Roc Hotel.

OTHER EVENTS

Thirty-seven sessions of committees, boards and other groups are scheduled. A complete list of committee and other organizational meetings will appear in the printed program.

The Annual Business Meeting will be held in the Napoleon Room on Sunday, June 12, from 12:00 noon to 12:30 p.m.

A Social Hour starting at 6:30 will immediately precede the Banquet on June 11.

THE PRINTED PROGRAM

Copies of the ADA program, containing abstracts of papers to be presented as well as those included by title, will be available at the registration desk at $1 each. A copy is being sent to all members at no cost in advance of the meeting. The aforementioned charge will be made for additional copies secured at the time of registration. Following is a list of papers to be delivered at the Scientific Sessions in the order of their presentation. Those included in the program by title are also listed for the information of readers of the Journal.

SCIENTIFIC SESSIONS

Saturday morning, June 11, 9:00 a.m.

FRANCIS D. W. LUKENS, Presiding

The "Dextrose-Deviates": Their Relationship to Subliminal Diabetes
LEO P. KRALL, HUGH L. C. WILKERSON and FRANK K. BUTLER, M.S. (by invitation), Joslin Clinic, Boston, Mass., and U.S. Public Health Service, Diabetes Field Research and Training Unit, Brighton, Mass.

Glucose Tolerance in Unselected Pregnancies
HUGH L. C. WILKERSON, U.S. Public Health Service, Diabetes Field Research and Training Unit, Brighton, Mass.

Studies of the Etiology and Significance of Abnormal Glucose Metabolism in Pregnancy
GEORGE W. WELSH, 3rd, University of Vermont College of Medicine, Burlington, Vt.

Glucose Tolerance and Cortisone-Glucose Tolerance in Subjects Whose Parents Are Both Diabetic
KELLY M. WEST, The University of Oklahoma Medical Center, Oklahoma City, Okla.

Prediabetes in an Identical Twin
J. M. PICKENS (by invitation), T. P. CHASE (by invitation), and ROBERT L. JACKSON, University of Missouri Medical Center, Columbia, Mo.

Changing Brittle to Stable Diabetes
THOMAS H. McGAVACK, George Washington University School of Medicine, Washington, D.C., and Intermediate Service, Veterans Administration Center, Martinsburg, W. Va.

What Happens to Juvenile Diabetics?
ABRAHAM H. KANTROW, State University of New York, Downstate Medical Center, Brooklyn, N.Y.

BANTING MEMORIAL LECTURE

Childhood Diabetes — Its Course, and Influence upon the Second and Third Generations
PRISCILLA WHITE, Joslin Clinic, New England Deaconess Hospital, Faulkner Hospital, Boston Lying-in Hospital, Boston Floating Hospital, New England Hospital, Assistant Professor of Pediatrics, Tufts University Medical School, Boston, Mass.

Saturday afternoon, June 11, 2:00 p.m.

HARVEY C. KNOWLES, JR., Presiding

The Effect of Denervation and Insulin on the Penetration of D-xylose into Rat Hemidiaphragms
MARIA GORDON BUSE (by invitation), and JOHN BUSE, Medical College of South Carolina, Charleston, S.C.

The Effects of Insulin in Vitro on Oxidative Reactions in the Krebs Cycle in Rat Adipose Tissue
ALBERT I. WINEGRAD and WALTER N. SHAW (by invitation), George S. Cox Medical Research Institute and the John Herr Musser Department of Research Medicine, University of Pennsylvania, Philadelphia, Pa.

Control by Insulin of the Magnitude of the Role of the Liver in the Disposition of a Glucose Load in Both Diabetic and Nondiabetic Dogs
LEONARD L. MADISON, BURTON COMBES (by invitation), and REUBEN ADAMS (by invitation), University of Texas Southwestern Medical School, Dallas, Tex.

Fatty Acid Biosynthesis in Liver from Alloxan Diabetic and Starved Rats
DAVID M. GIBSON (by invitation), JAMES ASHMORE, and DOROTHY D. HUBBARD (by invitation), Indiana University School of Medicine, Indianapolis, Ind.

Cholesterol Formation in Human Diabetics
LAURANCE W. KINSELL, GEORGE D. MICHAELS (by invitation), GEOFFREY WALKER (by invitation), and PAUL F. FLYNN (by invitation), Institute for Metabolic Research, Highland-Alameda County Hospital, Oakland, Calif.

Defective Synthesis of Triglycerides from C-14 Acetate in the Well-controlled Stable Diabetic
A. R. HENNES (by invitation), and Mr. T. W. REDDING (by invitation), Veterans Administration Hospital and University of Oklahoma Medical School, Oklahoma City, Okla.

The Evidence for Hormonal Role of Endogenous Glucagon Based on Direct Measurements by a Specific Immunoassay
ROGER H. UNGER, ANNA M. EISENTRAUT (by invitation), MARY S. McCALL (by invitation), and LEONARD L. MADISON, University of Texas Southwestern Medical School and Veterans Administration Hospital, Dallas, Tex.

Glucagon-induced Depression of Food Intake in Healthy Subjects: Another Possible Physiologic Role for Glucagon
S. B. PENICK (by invitation), and L. E. HINKLE, JR., New York Hospital-Cornell Medical Center, New York, N.Y.

Sunday morning, June 12, 9:00 a.m.
Clinical Session
FRANCIS D. W. LUKENS, Presiding

Motor Nerve Conduction Velocity in Diabetes Mellitus
THOMAS G. SKILLMAN (by invitation), ERNEST W. JOHNSON (by invitation), HELEN J. DRISKILL (by invitation), and GEORGE J. HAMWI, Ohio State University and University Hospital, Columbus, Ohio

Skin Temperature, Sweating, and Postural Blood Pressure in Diabetic Patients with Autonomic Neuropathy
GRACE M. ROTH (by invitation), DARRYL K. FOERSTER (by invitation), and RANDALL G. SPRAGUE, Mayo Clinic and Mayo Foundation, Rochester, Minn.

Carbohydrate Metabolism in Pregnancy: The Significance of Glycosuria
MILTON GROSS, Margaret Hague Maternity Hospital, Jersey City, N.J.

Serial Studies of Renal Function in Pregnancy Complicated by Diabetes Mellitus
ETHAN A. H. SIMS, University of Vermont College of Medicine, Burlington, Vt.

The Blood Ketone and Plasma Nonesterified Fatty Acid (NEFA) Concentrations in Diabetic and Normal Subjects
EMILE E. WERK, JR., and HARVEY C. KNOWLES, JR., Cincinnati Veterans Administration Hospital and University of Cincinnati College of Medicine, Cincinnati, Ohio

Vascular Lesion Common to Retinopathy and Nephropathy, as Well as Other Vascular Complications of Diabetes Mellitus
SIDNEY GOLDENBERG, MORRIS ALEX (by invitation), and HERMAN T. BLUMENTHAL (by invitation), The Jewish Hospital, St. Louis University School of Medicine, and Washington University School of Medicine, St. Louis, Mo.

Report on the Conference on Diabetic Retinopathy sponsored by the National Institute of Neurological Diseases and Blindness, Haddonfield, New Jersey, May 1-2
IRVING H. LEOPOLD, Chairman, Department of Ophthalmology, The Graduate School of Medicine, University of Pennsylvania, Philadelphia, Pa.

Episodic Hyperkalemic Paralysis: Report of a Case of Adynamia Episodica Hereditaria Associated with Diabetes Mellitus
HARVEY A. TRETBAR, University of Kansas School of Medicine, Lawrence-Kansas City, Kans., and Wesley Hospital, Wichita, Kans.; LEO CAWLEY (by invitation), University of Oklahoma School of Medicine, Oklahoma City, Okla., and Wesley Hospital, Wichita, Kans.

Sunday morning, June 12, 9:00 a.m.
Experimental Session
JAMES ASHMORE, Presiding

The Isolation of Rabbit Anti-Insulin Antibodies
E. R. ARQUILLA and C. COBLENCE, B.S. (by invitation), University of Southern California School of Medicine, Los Angeles, Calif.

The Use of Various Metabolic Parameters for the Assay of Insulin-like Activity with the Rat Epididymal Fat Pad
JACK R. LEONARDS and BERNARD R. LANDAU, Western Reserve University School of Medicine, Cleveland, Ohio

The Use of Adipose Tissue for the Measurement of Insulin-like Activity: Problems and Progress Report
ALBERT E. RENOLD, Baker Clinic Research Laboratory and Harvard Medical School, Boston, Mass.

Insulin Resistance with High Levels of Insulin-like Activity Demonstrable in Vitro and in Vivo Technics
J. C. SHIPP, J. STEINKE, R. O. RUSSELL (by invitation), and W. B. HADLEY, Joslin Clinic, Boston, Mass.

The Effect of Hypophyseal Stalk Section on Insulin Resistance
RICHARD A. FIELD (by invitation), WILLIAM A. HALL (by invitation), WILLIAM H. BAKER (by invitation), and DAGOBERTO SOSA (by invitation), Harvard Medical School and Massachusetts General Hospital, Boston, Mass.

An Insulin Antagonist Associated with Plasma Albumin
JOHN VALLANCE-OWEN and Miss M. DELORIS LILLEY (by invitation), King's College, University of Durham, and the Royal Victoria Infirmary, Newcastle upon Tyne, England.

The Role of Epinephrine as a Circulating Insulin Antagonist
J. J. GROEN, S. ELDER, M.Sc. (by invitation), E. HAVIVI, M.Sc. (by invitation), and I. CHOWERS (by invitation), Hadassah University Hospital and Hebrew University-Hadassah Medical School, Jerusalem, Israel

Changes in the Distribution of Endogenous Insulin between Liver and Peripheral Tissues during Physiologic Stimulation of Endogenous Insulin Secretion
LEONARD L. MADISON and WALTER SKINNER (by invitation), University of Texas Southwestern Medical School and Parkland Memorial Hospital, Dallas, Tex.

Glycoproteins in Newborns of Diabetic Mothers
O. V. SIREK, A. SIREK (by invitation), and B. S. LEIBEL, Banting and Best Department of Medical Research, University of Toronto Faculty of Medicine, Toronto, Ontario, Canada

Sunday afternoon, June 12, 2:00 p.m.
FRANKLIN B. PECK, SR., Presiding

The Diabetic Pancreas: A Re-evaluation Utilizing Refined Histologic Technics
BRUNO W. VOLK and SYDNEY S. LAZARUS, Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, Brooklyn, and Albert Einstein College of Medicine, Bronx, N.Y.

Diabetogenic Effects of Azacyclonol (Frenquel)
ARNOLD LAZAROW and BIRUTA TREIBERGS (by invitation), University of Minnesota Medical School, Minneapolis, Minn.

Tolerance for Glucose in the Partially Depancreatized Dog During Prolonged Administration of Tolbutamide
HENRY L. WILDBERGER and HENRY T. RICKETTS, University of Chicago, The School of Medicine, Chicago, Ill.

Effect of Chronic Tolbutamide Feeding on Pancreatic Function of Alloxan "Subdiabetic" Rats
J. W. WEBER (by invitation), J. P. COLOMBO (by invitation), D. KANAMEISHI (by invitation), R. GOLDBERG (by invitation), and PIERO P. FOA, Chicago Medical School, Chicago, Ill.

Lipoprotein Elevations of Diabetics on Tolbutamide as Compared to Lipoprotein Levels during Insulin Therapy: Preliminary Report
JOSEPH T. BEARDWOOD, JR., SIG-

MUND R. GREENBERG (by invitation), ROY G. KLOTZ, JR. (by invitation), Abington Memorial Hospital, Abington, Pa.

The Use of Long-acting Capsules of Phenformin (DBI) in Diabetic Therapy
JAMES M. MOSS, DeWITT E. DeLAWTER, SIDNEY A. TYROLER, and JAMES B. FIELD, Georgetown University Hospital, Washington, D.C.

Long-term Clinical and Laboratory Studies in Diabetic Patients Treated with Tolbutamide and Chlorpropamide
WILLIAM W. H. POTE, JR., ELMER A. ANDERSON (by invitation), BURT COCHRAN, JR., and ARTHUR J. RIESENFELD (by invitation), College of Medical Evangelists, Los Angeles, Calif.

Secondary Failure to the Sulfonylurea Drugs in the Treatment of Diabetes Mellitus
DeWITT E. DeLAWTER, JAMES M. MOSS and SIDNEY A. TYROLER, Georgetown University Hospital, Washington, D.C.

BY TITLE

Sulfonylureas: Their Clinical Boundaries in the Treatment of Diabetes Mellitus
ROBERT F. BRADLEY and LEO P. KRALL, Joslin Clinic, Boston, Mass.

Absent Deep Reflexes: A Diagnostic Clue in Unsuspected Diabetes
MAX ELLENBERG, The Mount Sinai Hospital, New York, N.Y.

Preliminary Studies of "Hard to Manage" Diabetics Treated with Long-acting Phenformin
GEORGINA FALUDI (by invitation), and LEWIS C. MILLS, Hahnemann Medical College and Hospital, Philadelphia, Pa.

Evidence of Insulin Resistance and Abnormalities in Glucose Utilization During Ether Anesthesia in Man
DOROTHY H. HENNEMAN (by invitation), and LEROY VANDAM (by invitation), Peter Bent Brigham Hospital and Harvard Medical School, Boston, Mass.

The Intravenous Tolbutamide Tolerance Test in the Evaluation of Abnormal Carbohydrate Metabolism
NORMAN M. KAPLAN (by invitation), USAF Hospital, USAF Aerospace Medical Center (ATC), Lackland Air Force Base, Tex.

Evaluation of Glucose Tolerance Tests and Nonesterified Fatty Acids in Diagnosis and Prediction of Diabetes Mellitus
THOMAS H. LAMBERT, ROY B. JOHNSON (by invitation), and GEOFFREY R. PAUL (by invitation), Scripps Clinic and Research Foundation, La Jolla, Calif.

The Early Treatment of Diabetic Acidosis in the Office
JAMES M. MOSS, Georgetown University School of Medicine and Georgetown University Hospital, Washington, D.C.

Routine Testing for Albumin in a Diabetic Clinic Using Uristix
JAMES M. MOSS, GEORGE E. SCHREINER (by invitation), and JOHN H. SWEENEY (by invitation), Georgetown University Hospital, Washington, D.C.

Experimental Diabetes in Fish
PAUL FOLEY NACE, McMaster University, Hamilton, Ontario, Canada, and Marine Biological Laboratory, Woods Hole, Mass.

Derelictions in Diabetes Detection
L. LEWIS PENNOCK, E. LOWELL BERRY (by invitation), PAUL FUGAZZOTTO (by invitation) and HOWARD P. CADWELL (by invitation), University of Pittsburgh School of Medicine and Allegheny County Health Department, Pittsburgh, Pa.

Clinical Outpatient Evaluation of the Usefulness of Oral Agents in the Treatment of Diabetes
WILLIAM W. H. POTE, JR., The College of Medical Evangelists, Los Angeles, Calif.

Long-term Clinical and Laboratory Studies in Diabetic Patients Treated with a New Form of Tolbutamide
WILLIAM W. H. POTE, JR., and GEORGE BARAT (by invitation), The College of Medical Evangelists, Los Angeles, Calif.

Observations on the Effects of Dexamethasone in Patients with Diabetes Mellitus and Retinitis Proliferans
WILLIAM W. H. POTE, JR., ALBERT J. JOSSELSON (by invitation), and DALE HAUCK (by invitation), The College of Medical Evangelists, Los Angeles, Calif.

Phenethylbiguanide: Comparative Pharmacology
ROBERT S. RADDING and STANLEY J. ZIMMERMAN (by invitation), Hermann Hospital, Houston, Tex.

Blindfold Study of Biphetamine in Obese Patients with Diabetes Mellitus
ARTHUR J. RIESENFELD (by invitation), and WILLIAM W. H. POTE, JR., The College of Medical Evangelists, Los Angeles, Calif.

The Rapid Intravenous Glucose Tolerance Test in Patients with Coronary Heart Disease
WILL G. RYAN (by invitation), and THEODORE B. SCHWARTZ, Presbyterian-St. Luke's Hospital and University of Illinois College of Medicine, Chicago, Ill.

Comparison of Small and Large Doses of Insulin in Therapy of Diabetic Acidosis
CHARLES E. SHAW, JR., GILBERT E. HURWITZ (by invitation), MORTON SCHMUKLER (by invitation), STUART H. BRAGER (by invitation), and SAMUEL P. BESSMAN (by invitation), University of Maryland School of Medicine, Baltimore

A Study of the Response to Chlorpropamide in Patients Requiring High Insulin Dosage
DAVID L. SINGER (by invitation), ROBERT C. STEWART (by invitation), and DAVID HURWITZ, Diabetes Clinic, Boston City Hospital, Boston, Mass.

The Use of the Sulfonylurea Hypoglycemic Drugs in Diabetes Mellitus
JUAN JOSÉ STAFFIERI, LUIS JORGE CARDONNET and DERIO EBERHARDT (by invitation), Sanatorio Britanico, Rosario, Argentina

Hyperinsulinemia in L-leucine Sensitive Hypoglycemia
SHIRLEY WEISENFELD and MARTIN G. GOLDNER, Jewish Chronic Disease Hospital, Brooklyn, N.Y.

Electron Microscopy of Islets of Langerhans in the Spontaneously Diabetic Chinese Hamster
JOSEPH R. WILLIAMSON (by invitation), and PAUL E. LACY, Washington University School of Medicine, St. Louis

POSTGRADUATE COURSE SERIES IN DIABETES AND BASIC METABOLIC PROBLEMS

The American Diabetes Association will hold its Ninth Postgraduate Course in New Orleans, Jan. 18, 19 and 20, 1961. The Scientific Sessions will be held in the Louisiana State University School of Medicine Auditorium, and the Jung Hotel will serve as headquarters.

On Wednesday, January 18, the program will be devoted to a presentation of the subject of "lipids" in depth, with Faculty Members drawn primarily from the Metabolism Study Section of the National Institutes of Health, which is meeting in New Orleans January 14-17.

A panel entitled "Diabetes as a Biological Spectrum" will be held at the close of the Thursday morning session to discuss the pathogenesis and manifestations of diabetes, and the pathophysiology of the diabetic state. "Seminars on Current Problems in Diabetes" will be held at the close of the Thursday afternoon session.

All inquiries pertaining to the Course should be directed to the national office, 1 East 45th Street, New York 17, N.Y.

The EIGHTH POSTGRADUATE COURSE offered by the American Diabetes Association was held in Los Angeles, California, Jan. 20, 21 and 22, 1960. Developed by the ADA Committee on Professional Education, the Course had as its Director T. S. Danowski, M.D., Pittsburgh, Committee Chairman. Arthur R. Colwell, Sr., M.D., Chicago, and Jerome W. Conn, M.D., Ann Arbor, were Vice Chairmen of the Committee. Roy F. Perkins, M.D., the Association's Governor for Southern California, was Chairman of the Local Committee. The Course was given in cooperation with the Schools of Medicine of The College of Medical Evangelists, University of California at Los Angeles and the University of Southern California.

A registration breakdown for the Eighth Postgraduate Course, and a comparison with the seven previous Courses, follow:

REGISTRANTS	1960 Los Angeles	1959 St. Louis	1958 Atlanta	1957 Columbus	1956 Dallas	1955 Philadelphia	1954 Rochester	1953 Toronto
Members	61	55	78	83	82	135	83	93
Membership applications pending	15	21	47	55	52	23	24	23
Nonmembers	73	31	53	57	47	18	17	61
Total	149	107	178	195	181	176	124	177
OTHERS ATTENDING								
Part-time registrants	3	2	1	1	—	2	—	—
*Graduate Students	—	—	—	—	—	1	†	†
*Fellows	7	7	1	—	2	3	†	†
*Residents	19	10	14	16	10	10	†	†
*Interns	6	8	4	8	4	5	†	†
*Medical Students	1	23	2	13	61	11	†	†
*Dietitians, Dietetic Interns	—	2	—	16	—	—	—	—
*Nutritionists	—	—	—	2	—	—	—	—
*Nurses	—	—	—	7	—	—	—	—
Councilors and Faculty	47	41	40	34	48	41	35	35
Diabetes Program Directors Workshop Participants	28	—	—	—	—	—	—	—
Insulin Bioassay Conference Participants	4	—	—	—	—	—	—	—
Other Guests	9	7	6	12	4	6	—	2
Total	124	100	68	109	129	79	35	37
Total of all categories	273	207	246	304	310	255	159	214
ADDITIONAL DATA								
Cancellations	9	8	19	12	12	15	25	5
Applications rejected due to limited facilities	—	—	—	—	—	—	47	92
Attendance at Banquet	200	173	245	200	324	246	208	236

*From metropolitan area of Course location.
†Graduate Students, Fellows, Residents, Interns and Medical Students were not formally registered in 1954 and 1953. A few were in attendance.

RESEARCH FELLOWSHIP AWARDS

The Committee on Research and Fellowships of the American Diabetes Association announces the award, for the 1960-1961 academic year (July 1, 1960-June 30, 1961), of the following Research Fellowships:

Adler Foundation Research Fellowship. David Norman, Ph.D., of Shrewsbury, Massachusetts, will complete the final year of the three-year Adler Foundation Research Fellowship. Dr. Norman will study by electronmicroscopy the changes occurring within the cell permitting sugar penetration of regions which, prior to treatment with insulin, were impenetrable. The subject material to be used for his work will be uncut vertebrate skeletal muscle, protoplasts of *Neurospora crassa*, and the giant amoeba, *Chaos chaos*. Dr. Norman will work with Professor Herschell K. Mitchell in the Department of Biology at the California Institute of Technology.

Bruce M. Carruthers, M.D., Philadelphia, Pennsylvania, will continue his work with Francis C. Wood, M.D., in the Department of Medicine, University of Pennsylvania, on studies of abnormalities in carbohydrate metabolism in patients with a congenital deficiency of red cell glucose-6-phosphate dehydrogenase.

Jurgen Steinke, M.D., Boston, Massachusetts, will continue his studies with George W. Thorn, M.D., and Albert E. Renold,

M.D., Baker Clinic Research Laboratory, Department of Medicine, Harvard University Medical School, on the problem of the interaction of insulin with other proteins, both in the pancreas and in blood serum.

John A. Colwell, M.D., Chicago, Illinois, will work with Arthur R. Colwell, Sr., M.D., in the Department of Medicine, Northwestern University Medical School, on the effects of various substances on insulin secretion in the unanesthetized dog.

Jack Kane Goldman, M.D., Pittsburgh, Pennsylvania, will work with George W. Thorn, M.D., and Albert E. Renold, M.D., in the Department of Medicine, Harvard University Medical School, Boston, Massachusetts, on the metabolism of mannose.

AVAILABILITY OF NEW FELLOWSHIPS

The Committee on Research and Fellowships will award three Research Fellowships for the academic year July 1, 1961—June 30, 1962. Deadline for applications is Nov. 15, 1960. Requests for application forms and other inquiries should be addressed to Mr. J. Richard Connelly, Executive Director, who will forward the information to the Committee.

4th IDF CONGRESS

The Fourth Congress of the International Diabetes Federation will be held July 10-14, 1961, in Geneva, Switzerland. Howard F. Root, M.D., Boston, Chairman; Alexander Marble, M.D., Boston; and Franklin B. Peck, Sr., M.D., Indianapolis, comprise the ADA Advisory Committee for the program. As information about the Congress becomes available it will be published in the Journal.

TEACHING AND RESEARCH IN DIABETES STILL AVAILABLE

The proceedings of the first National Conference on Teaching and Research in Diabetes are still available without cost by addressing your request to the American Diabetes Association, Inc., 1 East 45th Street, New York 17, N.Y.

The first National Conference was held in Atlantic City, New Jersey, May 3, 1958, under the sponsorship of the American Diabetes Association and the National Institute of Arthritis and Metabolic Diseases, Public Health Service, U. S. Department of Health, Education, and Welfare. The Conference Committee included E. Perry McCullagh, M.D., Chairman; Arthur R. Colwell, Sr., M.D.; John E. Howard, M.D.; Thomas McGavack, M.D.; and Robert H. Williams, M.D.

MAKE YOUR PLANS NOW FOR DIABETES WEEK

Among new material being prepared for use during Diabetes Week, November 13-19, is an informational pamphlet for service clubs who wish to participate in the ADA Detection Drive this year. Diabetes Week is customarily observed the week before Thanksgiving, and renews the annual Diabetes Detection Drive. Affiliates of the American Diabetes Association, County and State Medical Societies and other cooperating organizations are urged to develop their programs with these dates in mind.

NEW SCIENTIFIC EXHIBIT

"Everyday Problems in Diabetes: 201 Crystal-clear Answers" is the title of the new scientific exhibit being prepared by the American Diabetes Association for display at the American Medical Association Annual Meeting June 13-17, 1960, in Miami Beach, Florida. The exhibit will be in Space No. 326, Scientific Exhibit Section, Miami Beach Auditorium and Exhibition Hall.

The new exhibit was developed by the ADA Committee on Scientific Exhibits: Marshall I. Hewitt, M.D., Chairman; DeWitt E. DeLawter, M.D.; Alfred H. Free, Ph.D.; Robert C. Hardin, M.D.; William R. Kirtley, M.D.; Leo P. Krall, M.D.; C. J. O'Donovan, M.D.; George F. Schmitt, M.D., in collaboration with the Steering Committee of the Committee of Professional Education as follows: T. S. Danowski, M.D., Chairman; Arthur R. Colwell, Sr., M.D., Vice Chairman; Jerome W. Conn, M.D., Vice Chairman.

Approximately a year after its initial display, this exhibit will be added to the scientific exhibits already available on a loan basis to Clinical Societies of Affiliate Associations and to other medical organizations. These exhibits are as follows: "Diabetes — Today and Tomorrow: The Expanding Role of the Doctor," "Pathology of Diabetes," "Management of Diabetes Mellitus," "Vascular Complications of Diabetes," and "Diabetes Detection by the Physician."

NEW MEMBERS

The following were elected as of April 1 and May 1, 1960.

Active

California	
Brownson, Bradley C.	San Mateo
Huelskamp, Margaret	San Jose
Johnson, Aldis A.	Anaheim
Kadish, Arnold H.	Beverly Hills
Kritzer, Morton D.	Los Angeles
Reizman, Bert	Harbor City
Tranquada, Robert E.	Los Angeles
Young, Banff O.	Olive View
Florida	
Liebler, John B.	Coral Gables
Illinois	
Smith, Morton	Chicago
Indiana	
Rudesill, Robert L.	Indianapolis
Louisiana	
Munyan, Earl A., Jr.	New Orleans
Massachusetts	
Aarons, Jerome H.	Boston
Lakin, Mervyn H.	Medford
Shipp, Joseph C.	Boston
Steinke, Jurgen	Boston
Michigan	
Blackhurst, John F.	Midland
New Jersey	
Henneman, Philip H.	Jersey City
New York	
Mofenson, Howard C.	Mineola
Oldden, Jane B.	New York

Pennsylvania	
Allen, Wayne H.	Sayre
Texas	
Johnson, John E., Jr.	Galveston
Washington	
Ramquist, Reuel T.	Kirkland
Wisconsin	
Engbring, Norman H.	Milwaukee
Other Countries	
Canada	
Kovacs, Leslie L.	Montreal
West Germany	
Otto, Hellmut	Munster, Westring

NEWS OF AFFILIATE ASSOCIATIONS

The NEW JERSEY DIABETES ASSOCIATION will hold a joint meeting with the Section on Metabolism and Ophthalmology of the Medical Society of New Jersey on May 17, 1960, in Atlantic City. The following papers will be presented: "Ocular Manifestations of Diabetes," by Henry Abrams, M.D.; "The Role of the Ophthalmologist in Ocular Complications of Diabetes," by Robinson D. Harley, M.D.; "Trends in Surgical Treatment of Vascular Lesions in Diabetes," by Ralph Lev, M.D.; and "Management of Diabetes in the Patient with Visual, Renal and Neuropathic Complications," by Pauline R. Goger, M.D.

This Association, in cooperation with the New Jersey State Department of Health, held The Second Annual Spring Symposium entitled "Prediabetes and Diabetes in Pregnancy" at the Princeton Inn, Princeton, New Jersey, May 11, 1960. The program included: "Prediabetes in Pregnancy," by Hugh L. C. Wilkerson, M.D., Boston; "The Medical Management of Diabetes in Pregnancy," by Priscilla White, M.D., Boston; and "The Obstetrical Management of the Pregnant Diabetic," by Alan Guttmacher, M.D., New York. A panel discussion entitled "Prediabetes and Diabetes in Pregnancy," was held with Karl E. Paschkis, M.D., Philadelphia, as Moderator; Drs. Wilkerson, White and Guttmacher were panel members.

The NEW YORK DIABETES ASSOCIATION will hold a scientific and Annual Membership Meeting on May 17, 1960, at the Academy of Medicine in New York City. Papers on genetics and diabetes will be presented: "Basic Aspects," by Herman Chase, M.D., Providence, Rhode Island, and "Clinical Aspects," by Alexander Marble, M.D., Boston. The meeting will be conducted in cooperation with The Medical Genetics Committee of the American Eugenics Society.

NEWS NOTES

BRITISH DIABETIC ASSOCIATION FORMS MEDICAL AND SCIENTIFIC SECTION

Prof. F. G. Young, Cambridge, was named Chairman of the newly constituted Medical and Scientific Section of The British Diabetic Association at a meeting April 1 at Guy's Hospital, London. J. M. Stowers, M.D., Dundee, and D. A. Pyke, M.D., London, were elected Honorary Secretaries. The offices of the section are located with The British Diabetic Association at 152 Harley Street, London, W. 1.

PERSONALS

JAMES C. BRENEMAN, M.D., Galesburg, Michigan, has won a 1959 Ross Award for writing one of the "most significant scientific articles to be published in *GP* during the year," it was announced at the scientific program of The American Academy of General Practice in Convention Hall, Philadelphia, on March 23, 1960. The presentation of the $1,000 cash award was made by R. Varian Sloan, M.D., Honolulu, Chairman of the Academy's Ross Award Committee. Dr. Breneman's winning paper was titled: "Allergic Cystitis: The Cause of Nocturnal Enuresis."

ELLIOTT P. JOSLIN, M.D., Boston, Honorary President of the American Diabetes Association, was the principal speaker during the 168th Annual Meeting and Scientific Assembly of the Connecticut State Medical Society, April 26-28, 1960. Dr. Joslin spoke on "Diabetes in the Future" on April 27 in New Haven. He was introduced by William Mac Shepard, M.D., Putnam, President of the Connecticut Diabetes Association.

ARNOLD LAZAROW, M.D., PH.D., Minneapolis, will be the recipient of the Distinguished Service Award of the University of Chicago Medical Alumni Association at the Annual Reunion Banquet on June 8, in Chicago.

HENRY B. MULHOLLAND, M.D., Charlottesville, Virginia, has been honored by the establishment in his name of a Professorship in Internal Medicine at the University of Virginia School of Medicine. Dr. Mulholland, a past President of the American Diabetes Association, will be the first incumbent. In the fall, Dr. Mulholland will enter his thirty-eighth year on the faculty and his thirty-third year as Professor of Internal Medicine. A founder of the Virginia Council on Health and Medical Care, and President of the Medical Society of Virginia, Dr. Mulholland is Vice Chairman of the AMA Council on Medical Services and a consultant in several capacities to the U. S. Public Health Service. The new professorship will be known as The Henry B. Mulholland Chair.

HENRY T. RICKETTS, M.D., Chicago, T. S. DANOWSKI, M.D., Pittsburgh, and FRANCIS D. W. LUKENS, M.D., Philadelphia, participated in a panel discussion on diabetes at the One Hundred Sixty-second Annual Meeting of the Medical and Chirurgical Faculty of the State of Maryland, in Baltimore on April 21, 1960. Dr. Ricketts was moderator of the panel. Topics discussed included (1) Prediabetes, (2) Prevention of vascular complications, (3) Oral therapy, including indications and contraindications, tests of responsiveness, and the mode of action and clinical use of the sulfonylureas and of DBI, (4) Urine testing methods, and (5) The management of unstable diabetes.

"Diabetes — Today and Tomorrow: The Expanding Role of the Doctor," a scientific exhibit prepared by the American Diabetes Association was exhibited at the meeting by the Maryland Diabetes Association.